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

February 2026

Risk Assessment and Risk Management Plan for

DIR-221

Clinical trial of a genetically modified *Escherichia coli* for the treatment of ulcerative colitis

Applicant: Melius MicroBiomics Pty Ltd

Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR-221

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional release of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application has been prepared by the Regulator in accordance with the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of expert, agencies and authorities, and the public. The RARMP concluded that the proposed trial poses negligible to low 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 applicant, Melius MicroBiomics Pty Ltd (Melius), proposes to conduct a clinical trial to evaluate the safety and efficacy of a genetically modified (GM) *Escherichia coli* for the treatment of Australian patients with ulcerative colitis. Ulcerative colitis is a type of inflammatory bowel disease (IBD) that causes the formation of sores due to inflammation that affects the lining of the large intestine (colon) and rectum.

Clinical trials in Australia are conducted in accordance with requirements of the *Therapeutic Goods Act 1989*, which is administered by the Therapeutic Goods Administration (TGA). Therefore, in addition to approval by the Regulator, Melius would also require authorisation from TGA before the trial commences. Clinical trials conducted in Australia must also be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* and with the *Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Melius would also require approval from the Department of Agriculture, Fisheries and Forestry (DAFF) for import of the GMO into Australia.

The application

Project Title	Clinical trial with genetically modified *Escherichia Coli* for the treatment of ulcerative colitis.
Parent organism	*Escherichia coli* (Nissle strain).

Genetic modifications	*E. coli* has been modified by the:
	• Insertion of 2 copies of the tetrathionate reductase (*ttr*) operon from *Salmonella enterica* – survival advantage in inflammatory environment.
	• Deletion of 2 genes[1]
	  ○ Gene A - reduced survivability in the broader environment; and
	  ○ Gene B - potentially reduced ability to colonise the healthy gut compared to wild type (WT)

[1] Confidential Commercial Information: Some details about the modification in GM *E. coli* have been declared as Confidential Commercial Information under section 185 of the Act. This information was made available to the prescribed experts and agencies that were consulted on this application. CCI is not available to the public.

Principal purpose	The proposed trial is a Phase 1 study designed to evaluate the safety and efficacy of GM *E. coli*, for the treatment of Australian patients with ulcerative colitis.
Previous clinical trials	None, this is a first in human clinical trial.
Proposed limits and controls	
Proposed duration	5 years
Proposed release size	Up to 36 participants in Australia (including placebo).
Proposed locations	This clinical trial would be conducted within Australia at a hospital or clinical trial sites (medical facilities). The specific clinical trial sites are yet to be identified.
Proposed controls	• The GMO would be administered to trial participants within clinical trial sites. • Staff handling the GMO would be trained and would wear personal protective equipment. • Waste that may contain the GMO would be disposed of via the facility standard practices for disposal of biological waste. • Any unused doses of GMO would be disposed of at the clinical trial site at the end of the trial, in accordance with the *Transport, Storage and Disposal Guidelines*. • Participants would be instructed: ○ on appropriate hygiene practices, including proper hand washing procedures following toilet use. ○ to abstain from unprotected sex and to use a double barrier method. • The GMO would be transported and stored according to *Transport, Storage and Disposal Guidelines*.

Risk assessment

The risk assessment process considers how the genetic modification 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 risks are considered.

Credible pathways to potential harm that were considered include the potential exposure of people and animals to the GMO and the potential for transfer of genetic material to and from the GMO. The potential for the GMO to be released into the environment and its effects was also considered.

The risk assessment concludes that the proposed clinical trial poses negligible to low risks to human health and safety and negligible risks to the environment. Specific risk treatment measures are imposed to manage these risks to people.

Important factors in reaching the conclusions of the risk assessment included that:

- the parent organism has a long history of safe use as a probiotic,
- the GMO has selective replication in patients with inflammatory bowel disease,

- unintended exposure to the GMO would be minimised by the proposed limits and controls outlined in the risk management plan,
- bacterial infections usually self-resolve, but in some cases may need antibiotic or hospital treatment, and
- the likelihood of complementation and recombination of the GMO with other bacteria is unlikely to result in bacteria that is more pathogenic than the parent organism, and
- bacterial infections usually self-resolving, could be treated by antibiotics or may require specific hospital care in cases of severe infection.

Therefore, the Regulator considers that the dealings involved do not pose a significant risk to either people or the environment.

Risk management

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

The risk management plan concludes that the identified negligible to low risks can be managed to protect the health and safety of people by imposing specific risk treatment measures. Licence conditions are imposed to minimise the exposure of the GMO or novel bacteria to other people and the environment.

The licence includes limits on the number of trial participants and duration of the trial, as well as a range of controls to minimise the potential for the GMO to spread in the environment. 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.

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Abbreviations

AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
CTA	Clinical Trial Approval
CTN	Clinical Trial Notification
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings Involving Intentional Release
DNA	Deoxyribonucleic acid
EcN	*Escherichia coli* Nissle strain 1917
EU	European Union
FSANZ	Food Standards Australia New Zealand
GTTAC	Gene Technology Technical Advisory Committee
GM	Genetically modified
GMO	Genetically modified organism
HREC	Human Research Ethics Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICH-GCP	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
NSQHS	National Safety and Quality Health Service Standards
OGTR	Office of the Gene Technology Regulator
PPE	Personal Protective Equipment
PCR	Polymerase chain reaction
RAF	Risk Analysis Framework
RARMP	Risk Assessment and Risk Management Plan
SOP	Standard Operating Procedure
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
TGA	Therapeutic Goods Administration
TSDs	The Regulator's *Guidelines for Transport, Storage and Disposal*
USA	United States of America
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* (RAF) (OGTR, 2013b) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR website).

5. Figure 1 shows the information that is considered, within the regulatory framework above, 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 supply are assessed within this context. Chapter 1 describes 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. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the

Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. No public submissions were received.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry (DAFF).

9. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods. The TGA is responsible for administering the provisions of this legislation. Clinical trials of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, are also regulated by the TGA through the Clinical Trial Approval (CTA) scheme or the Clinical Trial Notification (CTN) scheme.

10. For clinical trials, the TGA has regulatory responsibility for the supply of unapproved therapeutic products. In terms of risk to individuals participating in a clinical trial, the TGA (as the primary regulatory agency), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) at each trial site all have roles in ensuring participants' safety under the *Therapeutic Goods Act 1989*. However, where the trial involves a GMO, authorisation is also required under gene technology legislation. To avoid duplication of regulatory oversight, and as risks to trial participants are addressed through the above mechanisms, the Regulator's focus is on assessing risks posed to people other than those participating in the clinical trial, and to the environment. This includes risks to people preparing and administering the GM bacteria, and risks associated with import, transport and disposal of the GMO.

11. The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects (ICH 1996). The guideline was developed with consideration of the current good clinical practices of the European Union (EU), Japan, and the United States of America (USA), as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). The TGA has adopted the ICH-GCP in principle as Note for Guidance on Good Clinical Practice (designated CPMP/ICH/135/95) (Therapeutic Goods Administration 2000), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

12. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). This document sets the Australian standard against which all research involving humans is reviewed. The *Therapeutic Goods Act 1989* requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.

13. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and GMO accounting and reconciliation.

14. DAFF administers Australian biosecurity conditions for the importation of biological products under the *Biosecurity Act 2015*. Biological products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GMOs).

15. Analysis of biological samples collected from trial participants administered with the GMO would occur at clinical trial sites, or at pathology laboratories. These facilities are regulated by State and Territory governments and adhere to professional standards for safety, disease control (Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019) and handling of pathology samples (National Pathology Accreditation Advisory Council; NPAAC).

16. The NPAAC advises Commonwealth, State and Territory health ministers on matters relating to the accreditation of pathology laboratories. NPAAC plays a key role in ensuring the quality of Australian pathology services and is responsible for the development and maintenance of standards and guidelines for pathology practices. The standards include safety precautions to protect the safety of workers from exposure to infectious microorganisms in pathology laboratories. While compliance with NPAAC standards and guidelines is not mandatory, there is a strong motivation for pathology services to comply, as Medicare benefits are only payable for pathology services if conducted in an appropriate Accredited Pathology Laboratory (APL) category, by an Approved Pathology Practitioner (APP) employed by an Approved Pathology Authority (APA). Accreditation of pathology services is overseen by Services Australia (formerly Department of Human Services), and currently, the only endorsed assessing body for pathology accreditation is the National Association of Testing Authorities (NATA).

17. The state and territory governments regulate hospitals and other medical facilities in Australia. All public and private hospitals and day procedure services need to be accredited to the National Safety and Quality Health Service (NSQHS) Standards developed by the Australian Commission on Safety and Quality in Healthcare (the Commission) and endorsed by the state and territory Health Ministers. The Commission coordinates accreditation processes via the Australian Health Service Safety and Quality Accreditation (AHSSQA) scheme. The NSQHS Standards provide a quality assurance mechanism that tests whether relevant systems are in place to ensure that the minimum standards of safety and quality are met. The safety aspects addressed by the NSQHS Standards include the safe use of sharps, disinfection, sterilisation and appropriate handling of potentially infectious substances. Additionally, the Commission has developed the National Model Clinical Guidance Framework, which is based on, and builds on NSQHS Standards to ensure that clinical governance systems are implemented effectively and to support better care for patients and consumers.

18. Hospitals and pathology laboratories, including their workers, managers and executives, all have a role in making the workplace safe and managing the risks associated with handling potentially infectious substances including the proposed GMO. There are minimum infection prevention practices that apply to all health care in any setting where health care is provided. These prevention practices were initially developed by the Centers for Disease Control and Prevention (CDC) and are known as the standard precautions for working with potentially infectious material. The standard precautions are described in the Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019).

Section 2 The proposed dealings

19. Melius is seeking authorisation to carry out a Phase 1 clinical trial to assess the safety and efficacy of a genetically modified (GM) *E. coli* that is modified to have a survival advantage in an inflammatory environment and a reduced survivability in the environment.

20. The dealings involved in the proposed clinical trial are:

(a) import the GMO;

(b) conduct the following experiments with the GMO:

i. prepare the GMO for administration to trial participants;

ii. administer the GMO to clinical trial participants by oral ingestion;

iii. collect samples from trial participants;

iv. analyse the samples;

(c) transport the GMO;

(d) dispose of the GMO;

and the possession (including storage), supply and use of the GMO for the purposes of, or in the course of, any of these dealings.

2.1 The proposed limits of the trial

21. The clinical trial is proposed to take place over a five-year period from the date of issue of the licence. Up to 36 participants in Australia would receive one to four doses of the GMO or a placebo via oral ingestion.

22. The clinical trial would take place at Mater Misericordiae Hospital Brisbane. Additional sites in Brisbane may be engaged for the recruitment of healthy volunteers if needed, but this is not anticipated by the applicant.

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

23. The applicant has proposed several controls to minimise exposure to the GMO, and to restrict the spread and persistence of the GMO in the environment. These include:

- Only trained personnel would conduct dealings with the GMO.
- Staff preparing the GMO would be required to wear appropriate PPE (e.g. gown and gloves) during the procedures.
- Although the GMO would be self-administered orally, staff present would be required to also wear appropriate PPE (e.g. gloves).
- Transport to and storage of the GMO at a clinical trial facility where it would be administered would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSDs).
- Disinfecting surfaces and equipment that come into contact with the GMO using an effective disinfectant (including, but not limited to, bleach 1000-5000 ppm; 70% ethanol or 1% Virkon™).

2.3 Details of the proposed dealings

2.3.1 Manufacturing of the GMO

24. The GMO would be manufactured in Canada and imported into Australia. The doses in the form of microbeads would be either packaged into blister packs, or in plastic vials or bottles composed of high-density polyethylene or equivalent materials. The applicant has stated that the type of primary packaging is still being finalised, and they would inform the OGTR when it has been finalised. The primary containers would then be enclosed within a secondary container of individual cartons with tamper-evident seals.

2.3.2 Transport and storage of the GMO

25. The GMO would be imported according to the packaging and labelling requirements of the International Air Transport Association (IATA) code UN3245.

26. Transport of the GMO from the Australian border would be directly to Mater Misericordiae Hospital Brisbane or other clinical trial sites if needed. The GMO would be packaged into blister packs, sealed plastic vials or bottles composed of high-density polyethylene or equivalent material. They would then be placed into a container, which would be enclosed within secondary packaging, consisting of cartons with tamper-evident seals.

27. Procedures would be in place to ensure that all transported GMOs can be accounted for, and that a loss of GMOs during transport can be detected; and access to the GMOs would be restricted to authorised

persons conducting dealings under the licence, who have been informed by the licence holder of any licence conditions that apply to them.

28. The proposed method of supply and storage of the GMO, as advised by the applicant, would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSD).

2.3.3 Clinical trial sites

29. The clinical trial would be carried out at a clinical trial site at the Mater Misericordiae Hospital, Brisbane. The applicant has also proposed that additional clinical trial sites in the Brisbane area may be used to recruit healthy volunteers if needed. Clinical trial sites would be assessed by the applicant for their ability to comply with local biosafety requirements. Clinical trial sites will need to meet the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practise (GCP) guidelines (ICH Guideline for Good Clinical Practice). Sites will also be selected based on their ability to comply with the TSDs and the licence conditions.

2.3.4 The clinical trial

30. The applicant proposes a Phase 1 study, which is to be conducted at clinical trial sites at the Mater Misericordiae Hospital, Brisbane, or other clinical trial sites in the Brisbane area (as noted in Section 2.3.3). The study aims to assess the safety and tolerability of the GMO.

31. The study would involve healthy participants and patients with ulcerative colitis who will receive either one dose or four oral weekly doses of the GMO or receive a placebo. All participants would be monitored for up to 28 days after final dose.

32. The study is proposed to occur in 2 stages, the first in healthy participants, before administration to patients with mild to moderate ulcerative colitis. All participants (healthy and patients with ulcerative colitis) would be enrolled in 3 cohorts of escalating doses (10^6 colony forming units (CFU); 10^7-10^8 CFU; and 10^9 CFU), with a placebo control.

2.3.5 Selection of trial participants

33. Inclusion criteria proposed by the applicant relevant to this assessment include that trial participants must:

- Be 18 to 75 years of age.
- Agree to use effective barrier contraceptives and abstain from unprotected anal sex for the duration of the trial.

34. Relevant exclusion criteria include participants who:

- Have diagnosis of any non-Inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections) within 3 months prior to randomisation.
- Use of probiotics within the 2 weeks prior to randomisation.
- Use of agents that alter gut transit time including laxatives, anti-diarrhoeal medications and diabetic or weight loss medications.
- Receipt of faecal microbiota transplantation (FMT) or other faecal-derived preparation within 6 months prior to randomisation.
- Use of antibiotics.
- Have previously had colectomy, ostomy, or other intestinal surgery (excluding cholecystectomy or appendicectomy).

35. In addition, participants may be excluded for any reason that, in the opinion of the investigator, makes the participant unsuitable for the study.

2.3.6 Preparation of the GMO for administration

36. The GMO would be in a microencapsulated form, as a bead. The doses of the GMO for administration would be prepared for dispensing as a bead in research pharmacies within the hospital or clinical trial sites by trained personnel. Access to the GMO during preparation would be restricted to the pharmacy personnel. Training would be provided by the licence holder in line with the licence conditions.

2.3.7 Oral administration of the GMO

37. The GMO would be self-administered orally (one bead) with water at clinical trial sites in the presence of a medical professional wearing gloves. Participants would then be instructed to wash their hands with soap and water after handling the bead. Administration of all doses would occur in the clinical trial sites.

2.3.8 Decontamination and disposal of the GMO

38. The applicant has stated that all decontamination of surfaces and spill management procedures would be conducted in accordance with the clinical trial site guidelines, OGTR requirements and biosafety training that would be provided to all personnel involved in dealings with the GMO.

39. Surface decontamination is proposed to occur before and after GMO handling and at the end of each working day with commonly used disinfectants at the appropriate contact time (e.g. 0.1% - 0.5% bleach; 70% ethanol and 1% Virkon™).

40. Any accidental spills or shedding of the GMO (e.g. rupture of samples containing the GMO, contaminated faeces or vomit,) is proposed to be immediately contained. The area would be isolated, and the material would be covered with absorbent material, followed by liberal application of disinfectants over the material and surrounding area for an appropriate minimum contact time for decontamination. Personnel cleaning up the area would be wearing the appropriate PPE (gloves, gowns, masks, and eye protection). Spill kits containing PPE, absorbent material and disinfectants would be maintained at all sites handling the GMOs and any incidents would be recorded and reviewed in accordance with the site's standard operating procedures and the licence conditions.

41. Any unused doses of the GMO would be disposed of in accordance with the clinical trial site biological waste guidelines and the TSDs at the end of the trial.

42. Participants would also be given instructions to wash their hands with soap and water following toilet use.

2.3.9 Sample collection and analysis

43. Samples would be collected at specified intervals for the duration of the clinical trial. Details of the types of samples and collection times have been declared commercial confidential information (CCI).

44. Samples would be collected at the clinic, and some sampling (not involving sharps) may also be carried out at home. Participants would be provided airtight containers and plastic sealable bags for home collected samples; and instructions on proper hand hygiene practices and appropriate storage of home collected samples. All contaminated waste from home-collected samples would also be double bagged and returned to the clinical trial site for decontamination.

45. Analysis of samples that may contain GMOs would occur in independent pathology laboratories.

2.3.10 Personal protective clothing

46. Clinical trial staff involved in the preparation and administration of the GMO to trial participants would wear gloves and lab coats.

47. In the clean-up of spills or shedding of the GMO (e.g. vomit, diarrhoea), the clinical trial staff would wear gloves, gowns, masks and eye protection.

2.3.11 Training

48. The applicant has indicated that staff handling the GMO during preparation and administration would be experienced research pharmacy personnel and medical professionals, respectively.

49. If the licence is issued, the applicant has stated that they would be responsible for ensuring all personnel handling the GMO would be trained in the licence conditions.

2.3.12 Accountability and Monitoring

50. The applicant has stated that procedures would be in place to ensure that all stored GMOs can be accounted for, and any loss of the GMO can be detected.

51. Participants would be monitored for any adverse events following the administration of the GMO and during the follow-up visits to the clinical trial sites.

2.3.13 Contingency plans

52. In the event of unintentional release of the GMO due to spills or shedding of the GMO, personnel would be instructed to follow spill management procedures, including:

(a) the GMO will be contained to prevent further dispersal;

(b) persons cleaning up the GMO will wear PPE including gloves, gown, masks and eye protection;

(c) the exposed area will be decontaminated with an appropriate chemical disinfectant effective against the GMO;

(d) any material used to clean up the spill or PPE worn during the clean up will be decontaminated;

(e) clinical trial staff will notify the licence holder as soon as reasonably practicable; and

(f) the licence holder will notify the Regulator as soon as reasonably practicable.

53. In the event of exposure of people to the GMO via inhalation, direct contact with facial mucosa, or ingestion, the applicant proposes such persons would be instructed to:

(a) rinse their eyes, nose, and mouth thoroughly with water;

(b) monitor for any gastrointestinal discomfort; and

(c) report the incident to the licence holder, the institution's IBC and the trial sponsor.

Section 3 Parent organism

54. The GMO is derived from the bacterium *Escherichia coli* Nissle 1917 strain. It is a member of the genus *Escherichia* in the family and the family Enterobacteriaceae. It meets the criteria to be classified as a Risk Group 1 organism in accordance with the Australian/New Zealand Standard 2243.3:2022 (Standards Australia/New Zealand, 2022). The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of *E. coli* Nissle strain will be discussed here.

55. The classification *Escherichia* has been complex, but it is mainly classified to 7 species (*E. coli*, *E. albertii*, *E. fergusonii*, *E. hermannii*, *E. marmotae*, *E. ruysiae* and E. *whittamii*) (Meier-Kolthoff et al., 2014; Cobo-Simón et al., 2023). The species *E. coli* is further characterised into 14 phylogenetic groups (A, B1, B2-1, B2-2, C, D1, D2, D3, E1, E2, F, G, Shig1 and Shig2), which includes the genus of *Shigella* as it has been shown to be a subspecies of *E. coli* (Abram et al., 2021).

56. *E. coli* was first described by Theodor Escherich in 1885 (Lim et al., 2010). *E. coli* are facultative anaerobic, gram negative, non-sporulating rod shaped bacteria. Facultative anaerobes can survive both in aerobic as well as in anaerobic conditions. *E. coli* can be either non-motile or motile, with a flagellum, and grow best at 37°C. *E. coli* can either live inside a host or in the environment. Inside a host, *E. coli* can either be commensal or pathogenic.

3.1 Commensal *E. coli*

57. *E. coli* usually has a commensal relationship with the host, deriving a steady supply of nutrients as well as protection and dissemination from the host. This interaction, however, provides some benefits for the host as *E. coli* microbiota prevent colonisation by and growth of pathogens, by producing bacteriocins and other mechanisms (Rastegarlari et al., 1990; Vollaard and Clasener, 1994; Hudault et al., 2001; Schamberger et al., 2004). *E. coli* has a wide host range, colonising mammals, birds, reptiles and amphibians (Berg et al., 1983).

58. Commensal *E. coli* strains are found in the large intestine, especially in the caecum and the colon, mainly in the mucus layer covering the epithelial cells throughout the tract. They are shed into the intestinal lumen with degraded mucus components and are excreted in the faeces. It is estimated that there are 10^7-10^9 *E. coli* in each gram of human faeces (Tenaillon et al., 2010). *E. coli* has adapted to its ecological niche and competes with other bacteria for nutrients (Poulsen et al., 1994; Licht et al., 1999; Rang et al., 1999).

3.2 Pathogenic *E. coli*

59. Although most strains of *E. coli* are non-pathogenic and are commensal residents of the human gut (Gordon and Cowling, 2003), some can cause diseases. *E. coli* is estimated to cause hundreds of thousands of deaths a year (Russo and Johnson, 2003). Pathogenic *E. coli* have virulence factors that are not present in commensal *E. coli*, such as toxins, adhesins, protective coats and invasins.

60. Pathogenic *E. coli* strains causing infection within the gut can be classified based on the symptoms they cause such as enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC) and enteroaggregative *E. coli* (EAEC) (Vila et al., 2016). Most commonly, infections with these *E. coli* cause diarrhoea or gastroenteritis and are often acquired though eating contaminated food. Some EHEC have a virulence factor that leads to the production of a toxin called shiga, so they are also called shiga toxin-producing *E. coli* (STEC). STEC infections can cause bloody diarrhoea, abdominal cramps, vomiting and sometimes a serious condition called haemolytic uraemic syndrome which can be fatal (Lim et al., 2010). Large outbreaks of STEC sometimes occur in developed countries but are relatively uncommon in Australia. 822 STEC infections were notified in Australia between 2000 and 2010 along with 169 cases of haemolytic uraemic syndrome (Vally et al., 2012). Antibiotics are not recommended for STEC infections and may be harmful (2018).

61. Pathogenic *E. coli* can sometimes cause disease outside of the gut and are therefore called extraintestinal pathogenic *E. coli* (ExPEC). These *E. coli* often colonise the human gut without causing issues but become a problem when they are able to spread to other body sites. Some of the same virulence factors (such as P fimbriae and specific capsules) that make these *E. coli* damaging when they are outside of the gut, help them to successfully colonise the human gut (Vila et al., 2016).

62. ExPEC are the most common cause of urinary tract infections (UTIs) and are sometimes called urinary pathogenic *E. coli* (UPEC). UTIs include infection of the bladder, urethra, ureters and kidneys. They are normally treated with antibiotics, but if left untreated (or if the antibiotics used are ineffective) can lead to serious complications (healthdirect, 2020). *E. coli* that cause UTIs have multiple virulence factors; adhesins that help them stick to cells, toxins that help them spread into tissues and evade the immune system, the ability to form biofilms, and iron acquisition mechanisms that help them get nutrients (Vila et al., 2016).

63. A large study into skin and soft tissue infections found *E. coli* was the third most common cause of infection (*Staphylococcus aureus* was the most common cause) (Moet et al., 2007). When these infections are not self-limiting, they are treated with antibiotics to ensure the infection does not spread or enter the bloodstream.

64. Under certain conditions, including after surgical operations or immunosuppression, previously commensal *E. coli* can act pathogenically. Bloodstream infections are the most common and life-threating complication after solid organ transplants, and about 37% of these are caused by *E. coli (AAP, 2018)*.

3.3 Free-living *E. coli*

65. It is estimated that half of the *E. coli* population resides in water and sediments (Savageau, 1983). The oral – faecal route is the main mode of transmission and distribution of *E. coli* and its presence in water is often used as an indicator of faecal pollution (Savageau, 1983; Russell and Jarvis, 2001). However, more recent reports show that some *E. coli* are naturalised to soil, sand and sediments (Jang et al., 2017).

3.4 *E. coli* Nissle strain

66. The parent organism *E. coli* Nissle strain (EcN) was first isolated by Alfred Nissle in 1917 and is a commensal and non-pathogenic strain of *E. coli* that belongs to the B2 phylogenetic group (Sonnenborn, 2016; Wassenaar, 2016). Although the B2 phylogenetic group is typically associated with *E. coli* strains that can cause disease, unlike pathogenic strains, EcN does not express disease-causing factors and is not known to contain any conjugative plasmids (circular DNA that is separate from the chromosome) or antibiotic resistant genes (Grozdanov et al., 2004; Nowrouzian et al., 2005). It is reported to contain 2 cryptic plasmids pMUT1 and pMUT2 that have unknown functions, but have been used as a detection method for EcN (Blum-Oehler et al., 2003). Exposure to human blood serum can kill EcN, and hence it is easily cleared by the immune system (Grozdanov et al., 2002).

67. EcN is the most frequently used probiotic *E. coli* strain. It is commercially available as an over-the-counter probiotic in capsules and is mostly used to treat inflammatory bowel disease. In Australia, EcN (Mutaflor®) is a registered complementary medicine with the TGA. The maximum recommended daily dose is 10^{11} CFU, and treatment is usually well tolerated and does not cause significant changes to stools in healthy people but can reduce constipation. Some ingested bacteria pass through the gut rapidly, whereas bacteria that live in the gut for a significant amount of time are considered to have colonised the gut. A systematic review of multiple studies using the EcN strain suggests that it is not very efficient at colonising the adult human gut long term (Wassenaar, 2016). Another review has also reported that the EcN can maintain its colonisation of healthy adult mice and humans for up to 24 weeks after the last treatment, but the majority of individuals clear EcN in 2 weeks (Gurbatri et al., 2024). However, other reports claim it is a good coloniser (Lodinová-Zádniková and Sonnenborn, 1997; Lasaro et al., 2009).

68. EcN has also been used in studies for treatment of diarrhoea and urinary tract infections in dogs, pigs, calves; as a probiotic against other pathogenic bacteria in chicks; and to improve the immunity and egg laying performance of Japanese quails. No serious adverse events or toxicity were identified in these animals (von Buenau et al., 2005; Mourand et al., 2021; Helmy Yosra et al., 2022; Rudinsky et al., 2023; Wu et al., 2023; Sedaghat et al., 2025).

69. EcN has been shown to directly inhibit the growth of various pathogenic bacteria by the secretion of various antimicrobial molecules and enzymes (e.g. *Escherichia spp., Salmonella spp., Klebsiella spp., Shigella spp., Pseudomonas spp., Staphylococcus spp.*). It can also prevent biofilm formation by other EHEC pathogens, *Salmonella enterica*, *P. aeruginosa, Staphylococcus aureus* and *S. epidermidis*. Bacteria that form biofilms are highly tolerant of external stresses such as antibacterial agents and are the major cause of chronic and medical device related infections (Sassone-Corsi et al., 2016; Sonnenborn, 2016; Fang et al., 2018; Chen et al., 2023).

70. EcN has anti-inflammatory effects in the intestinal epithelial cells (IEC) in inflammatory bowel disease (IBD). A review by Chen et. al., has suggested that EcN does this by competing against harmful bacteria for resources; stimulating IECs to produce various molecules to resist pathogenic bacteria and inhibit the expression of pro-inflammatory molecules; and stimulating IECs to repair the intestinal epithelial barrier (Chen et al., 2023).

71. It has recently been reported that *E. coli* belonging to the B2 phylogenetic group (including EcN), contains polyketide synthase (*pks*) islands that encode colibactin, a genotoxin (Auvray et al., 2021). The *E. coli pks* island is encoded by 19 genes (*clbA* – *clbS*) (Auvray et al., 2021). Colibactin is reported to induce double stranded breaks and chromosomal mutations (Falzone et al., 2024). Some *in vitro* and *in vivo* laboratory studies have demonstrated that EcN is able to cause mutations (Pleguezuelos-Manzano et al., 2020; Rosendahl Huber et al., 2024). Studies have also shown that the deletion of *clbA* gene from EcN, is

able abrogate its ability to cause DNA damage. However, this deletion impairs its probiotic activity (Olier et al., 2012). Conversely, other studies have demonstrated that EcN does not cause/induce genotoxicity (Janosch et al., 2019; Dubbert et al., 2020). Results of *in vitro* and mouse preclinical studies suggest that the genotoxicity of colibactin is associated with the expression of adhesion molecules FimH and FmlH. These studies demonstrated that EcN did not exacerbate colorectal cancer (CRC) development in a CRC mouse model when compared to another *pks*$^{+}$ *E. coli* strain isolated human colorectal cancer (*E. coli* 11G5, also known as *E. coli* CCR20). The difference between the strains was attributed to the variation in the genotype of FimH, where EcN expresses a genotype of FimH that is found in avirulent commensal strains of *E. coli* such as the K12 strain (Jans et al., 2024). No information was found that shows that EcN can cause cancer in humans.

3.5 Genetics of *E. coli*

72. The genome size varies widely across *E. coli* strains, with the average genome containing around 5000 genes. Only 1700 genes are conserved among all strains (these are commonly referred to as 'strict core') and 3000 genes are conserved in at least 95% of the strains (commonly referred to as 'soft core') (Kaas et al., 2012). Hence each strain contains genes from the core genome and genes from an extended pool of approximately 8000 genes. This provides a high level of plasticity in the genome and also reflects the adaptive nature of the organism (Tenaillon et al., 2010).

73. The NCBI RefSeq and NCBI databases predict that EcN has 5126 and 5409 genes, respectively (National Centre for Biotechnology Information, 2025). As mentioned in Section 3.4, EcN is not known to have any native conjugative plasmids or genes that carry resistance to antibiotics.

3.5.1 Horizontal gene transfer (HGT)

74. In addition to a large gene pool, *E. coli* can exchange genetic elements with other bacteria present in the surrounding environment. Genetic elements are thought to move horizontally (to compatible bacteria) and vertically (to offspring) as they can help bacteria adapt to changing environments (Kaper et al., 1995) and contribute to the development of novel strains and pathotypes.

75. *E. coli* carry genetic material in chromosomes and plasmids. Chromosomes contain the essential genetic material of *E. coli* and are generally vertically inherited by the offspring from the parent. Plasmids are usually smaller packets of DNA that exist separately from the bacterial chromosomes and can replicate independently of chromosomes. Enterobacterales, which include *E. coli*, often carry multiple plasmids simultaneously (Garcia et al., 2007; Dionisio et al., 2019).

76. There are four main genetic mechanisms that enable the horizontal transfer of genetic elements in *E. coli*: conjugation, transformation, transposition and transduction (See Figure 3).

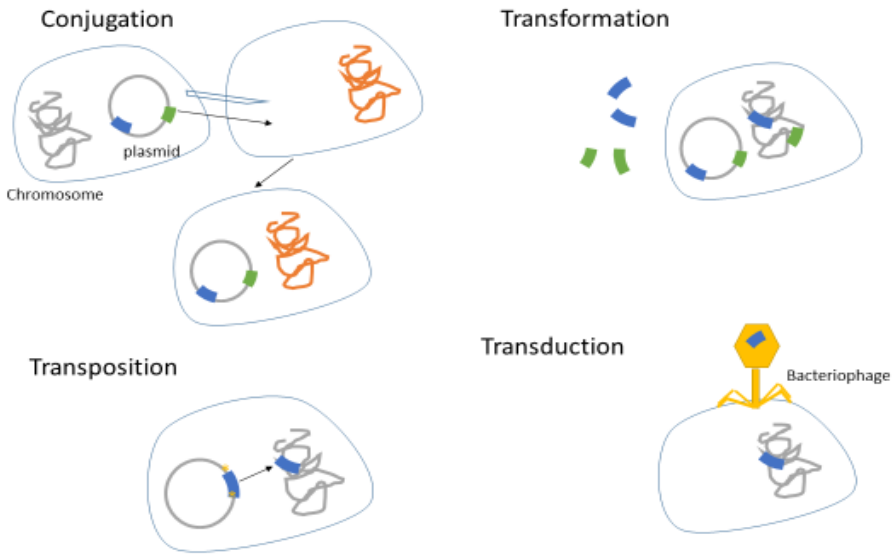

Figure 3. Common mechanisms of horizontal gene transfer in bacteria

77. **Conjugation** describes the direct transfer of DNA from one bacteria to another and is arguably the most important mechanism of horizontal gene transfer in bacteria (Sørensen et al., 2005; Norman et al., 2009). It involves 2 bacteria coming into physical contact and forming a mating pair. The donor bacterium produces a filamentous pilus that allows a copy of the plasmid to travel across into the recipient bacterium Both the donor and acceptor now have a copy of the plasmid.

78. **Transformation** in *E. coli* involves the induction of competence, DNA binding followed by fragmentation of the DNA, uptake and stable maintenance of the DNA by either integration in the genome (recombination) or recircularisation of plasmid DNA (Sørensen et al., 2005; Harrison and Brockhurst, 2012).

79. **Transduction** is the movement of genetic material with the help of bacteriophages. Erroneously packed host DNA can be transferred to other bacteria upon its infection with the phage. In theory, any region of the bacterial genome can be transferred in that way, including plasmids, but the DNA will not be retained by the host unless the phage integrates into the bacterial genome (prophage). The regions co-integrated with prophage DNA are commonly the flanking regions of the prophage insertion site (Berg et al., 1983).

80. **Transposition** describes the translocation of a discrete segment of DNA (the transposable element or transposon) from a donor site to non-homologous target sites. Transposable elements encode the machinery required to execute such rearrangements in addition to other determinants such as antibiotic resistance genes and genes for virulence factors. In general, transposition is an infrequent event probably because of its capacity for deleterious effects in the host. Usually, a transposon is translocated onto a plasmid upon conjugation. This may be followed by the integration of the transposon into the chromosome. For many transposons, however, plasmids rather than the bacterial chromosome appear to be the preferred target (Craig, 2014).

81. Laboratory studies have shown that HGT can occur in EcN both *in vitro* and *in vivo* (Zebrafish and mice) (Frazão et al., 2019; Fang et al., 2024).

3.6 Bio-distribution and shedding

82. The principal route by which the GM bacteria may enter the wider environment following inoculation is via shedding. Further, GM bacteria could also enter the environment via dispersal of unused GMO beads.

83. Human faeces is estimated to contain about 10^{12} bacteria per gram (Sender et al., 2016) and healthy adults produce in the order of 100 g of faeces per day in western countries (Cummings et al., 1992). So approximately 10^{14} bacteria per person per day may enter sewage. Around 90% of these bacteria will belong to the Firmicutes (also called Bacillota) and Bacteroidetes (also called Bacteroidota) phyla (Rinninella et al., 2019). It has been estimated that there are about 10^{8} CFU of *E.coli* per gram of faeces (Zuo et al., 2011) or 10^{7}-10^{9} *E.coli g/faeces* (Tenaillon et al., 2010).

84. Human gut microbiota is excreted into sewage and wastewater, where it undergoes standard waste treatment processes (which can vary significantly), prior to the water being released back into the environment. Sewage treatment is likely to be effective at removing the GM bacteria from sewage. However, due to variable levels of sewage treatment in the wastewater plants (Toze et al., 2012), this could result in varying amounts of bacteria remaining in the sewage and could result in dispersal of some microbiota, including GM bacteria, directly into rivers or marine environments.

85. Bacterial populations in raw sewage include human faecal bacteria, bacteria resident in the sewer system infrastructure, and environmental bacteria originating from grey water and surface runoff (Shanks et al., 2013). In untreated sewage samples collected from 13 wastewater treatment plants in the United States, the most abundant bacterial phyla were Proteobacteria (also called Pseudomonadota), which includes *E. coli* (average 62%), Firmicutes (average 21%) and Bacteroidetes (average 13%) (Shanks et al., 2013). Similarly, in activated sludge samples collected from 14 wastewater treatment plants in east Asia and North America, the most abundant phyla were Proteobacteria (35-65%), Firmicutes (averaging 8%), Bacteroidetes (averaging 7%) and Actinobacteria (averaging 7%) (Zhang et al., 2012).

86. In urban areas, most wastewater is processed at centralised wastewater treatment plants (WWTPs). Processes in WWTPs vary but generally the wastewater undergoes a primary treatment process involving sedimentation, followed by a secondary treatment where aeration is used to allow bacteria to digest organic matter. Some, but not all, WWTPs use tertiary treatment to disinfect the water further via chlorination, ozonation, UV treatment or other methods. After treatment, most wastewater is returned to the ocean, a lake, or a river. A UK study of 162 WWTPs found that primary treatment did not reduce the concentration of faecal indicator bacteria much, but secondary treatment reduced faecal indicator bacteria by 95-99%, and tertiary treatments reduce this by a further 93-97% (Kay et al., 2008). Overall, this is a reduction in bacteria of up to 3000-fold.

87. In 2022-2023, approximately 58% of urban sewage in Australia underwent tertiary wastewater treatment (Bureau of Meteorology, 2024) . A small proportion of Australian urban sewage, for example in inland towns, may only undergo secondary wastewater treatment prior to effluent discharge into inland waters (Water Quality Australia Sewerage System Guidelines website, accessed 10/11/2025). Untreated sewage is sometimes released from urban sewage systems due to overflow events, particularly during wet weather. In 2022-2023, the volume of wastewater losses and spills in Australia was approximately 3.5% of total wastewater collected (Bureau of Meteorology, 2024). Some sewage overflows enter the ocean, where the GMO is not expected to survive, but other sewage overflows occur on land or enter inland waters and could release live GMO.

88. Urban sewage treated at a wastewater treatment plant produces biosolids as well as effluent. In 2023, about 85% of biosolids produced in Australia were reused, including about 79.3% that were applied as fertiliser to agricultural land (Australian Biosolids Statistics website, accessed 10/11/2025). Roughly half of biosolids for reuse are treated to grade A level, which involves almost complete pathogen kill, and the other half are treated to grade B level, which involves a significant reduction in pathogens (Darvodelsky, 2012). Grade A biosolids would not contain live GMO, however, some GMO could survive in Grade B biosolids, which typically achieve a 1.5-2 log reduction in microorganism concentrations compared with raw sewage solids (Department of Environment and Science, 2019). In Queensland, the use of biosolids is regulated under the End of Waste (EOW) code for Biosolids (ENEW07359617) issued by the Queensland Government in accordance with section 159 of the *Waste Reduction and Recycling Act 2011*. Biosolids must meet the requirements of <100 most probable number (MPN) of *E. coli* per gram of dry weight for it to be used.

89. An analysis of raw and treated wastewater from 4 wastewater treatments plants across Australia found an average of 126 different genera of bacteria were present (Ahmed et al., 2017). The 10 most abundant genera were *Pseudomonas, Arcobacter, Bacteroides, Paludibacterium, Conchiformibius, Flavobacterium, Polynucleobacter, Acinetobacter, Parabacteroides,* and *Cloacibacterium.* A study of 4 WWTPs in Queensland found that human pathogenic *E.coli* could sometimes survive tertiary treatment and reach the environment (Anastasi et al., 2010). Determining the number of *E.coli* in the environment that came from waste water is complicated by birds and other animals carrying similar *E.coli* strains to humans (Anastasi et al., 2012).

90. Some human waste does not enter commercial wastewater treatment but is instead subject to various types of on-site treatment. These include septic systems, aerated wastewater treatment systems and dry composting toilets. Generally, these treatments are less effective at killing bacteria compared to wastewater treatment plants.

3.7 Control, environmental stability and decontamination methods

91. EcN is sensitive to any broad-spectrum antibiotics against gram-negative bacteria (Sonnenborn and Schulze, 2009). In most cases, people will recover without the use of antibiotics.

92. *E. coli* can survive in the environment (soil, manure, water) for periods ranging from weeks to a year (van Elsas et al., 2011). Persistence in the environment depends on various factors such as availability of nutrients, temperature, oxygen and pH (van Elsas et al., 2011). Conditions in the gut would be more

favourable to the persistence of *E. coli* than those in the broader environment due to the larger variability of the factors that affect persistence in the broader environment (Petersen and Hubbart, 2020).

93. All bacteria can be killed by autoclaving or high-temperature incineration (Rutala et al., 2008). Ethanol (60-80%), formaldehyde (4%) and Virkon (1%) are effective disinfectants for vegetative bacteria, but they lack sporicidal action or require long contact time (2 – 20 hours for tested species) to kill bacterial spores. Hypochlorite (0.5%) kills both vegetative bacteria and spores within 10 minutes contact time but is less effective in the presence of organic matter (Russell, 1990; Rutala et al., 2008). Methods of decontamination effective against the parent organism, EcN, are expected to be equally effective against the GMO.

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

94. The GMO is based on EcN and has been genetically modified to enable it to colonise the inflamed gut and to reduce its ability to survive in the environment. The GMO is designed to treat patients with ulcerative colitis, which is a form of inflammatory bowel disease (IBD).

95. The GMO has been modified by the insertion of the tetrathionate reductase (*ttr*) operon to provide a survival advantage in inflammatory environment and by the deletion of 2 genes (Gene A and Gene B – details have been declared CCI) to reduce its ability to survive outside the gut. The deletion of Gene B may also result in reduced ability to colonise a healthy gut. Further details of the genes are described in sections below.

4.1 Tetrathionate reductase operon

96. The tetrathionate reductase (*ttr*) operon from *Salmonella enterica* serovar Typhimurium is inserted into the region encoding Gene B of EcN to generate the GMO. The *ttr* operon encodes the structural and regulatory genes (ttrA, B, C, S and R proteins) that form the tetrathionate reductase enzyme complex, to allow bacteria in the genus *Salmonella, Proteus* and *Citrobacter* to use tetrathionate in anaerobic conditions (Hensel et al., 1999). The ability to use tetrathionate under inflammatory and anaerobic conditions provides a growth advantage in *S. enterica* compared to other gut microbiota (Winter and Bäumler, 2011).

97. Recently, the *ttr* operon has also been isolated in a novel strain of *E. coli* (Adsit et al., 2022). It was deduced that the *ttr* operon in this novel *E. coli* was likely of *Citrobacter* lineage, acquired through horizontal transfer and likely chromosomal (Adsit et al., 2022). The study also determined that the *ttr* operon is present in approximately 0.7% of the *E. coli* genomes in the National Centre for Biotechnology Information (NCBI) database. This suggests that *ttr* operon is becoming established in the *E. coli* population (Adsit et al., 2022).

4.2 Other genes

98. As with most biological organisms, there are various critical enzymes in *E. coli* that are involved in metabolism and biosynthesis pathways, DNA replication and the generation of essential molecules for the survival of *E. coli*. The GMO contains deletion of 2 genes (Gene A and Gene B) encoding enzymes in EcN. Deletion of Gene A reduces its ability to survive outside the gut. Deletion of Gene B may reduce its ability to colonise a healthy gut compared to WT in mouse studies. Details of these modifications have been declared as CCI. This information was made available to the prescribed experts and agencies that were consulted on this application.

4.3 Characterisation of the GMO

4.3.1 Genetic stability and molecular characterisation

99. The applicant has stated that the glycerol stocks of the GMO have remained stable for at least 10 years when stored at -80°C, as determined by genetic sequence analysis. The master cell bank (MCB) used to generate the working cell bank (WCB) is tested annually for genomic stability.

100. However, there have been no studies of the genetic stability of the GMO under repeat passaging conditions. A study investigating the stability of the parent EcN strain during a scale up manufacturing process (continuous passaging of 140-160 generations) demonstrated that the mutational hotspot to be within the pMUT plasmid, which has been modified to express different proteins (Munkler et al., 2024). The native cryptic plasmids pMUT1 and pMUT2 are only found in EcN and have been reported to be stable within EcN both *in vitro* and *in vivo* (Sonnenborn and Schulze, 2009; Ou et al., 2016; Kan et al., 2021). Generally, these mutations lead to the inactivation of the protein, as it is a metabolic burden to EcN (Munkler et al., 2024). The GMO does not contain any modified plasmids, and all the genetic modifications were carried out in the chromosome of EcN and hence likely to be more genetically stable.

101. EcN has also been shown to be stable through 100 serial passages *in vitro*, and in newborn children for 24 months; to not pick up plasmids that contain virulence factors (IncFI and IncFII types); and to not take up phage-encoded genetic information for the production of Shiga-like toxins (Sonnenborn and Schulze, 2009).

102. As mentioned in Section 3.5.1, horizontal gene transfer can occur via transduction through bacteriophages integrating into the bacterial chromosome via prophage attachment sites. The bacteriophage attachment site used to insert the *ttr* operon into the GMO has been deleted and this is likely to result in increased stability of the inserted gene from further modification/integration by bacteriophage.

4.3.2 Stability in the environment and decontamination

103. The stability of this GMO in the environment (surfaces, water types and sediments) has not been tested. Methods of decontamination effective against the parent organism, EcN, are expected to be equally effective against the GMO (see Chapter 1, Section 3.7).

4.3.3 Pre-clinical studies using the GMO

104. Pre-clinical studies were carried out in healthy mice and colitis-prone mice ($Muc^{-/-}$ mice) using WT EcN and the GMO (Verdugo-Meza et al., 2024). In this pre-print publication, it was demonstrated that the GMO persists in $Muc^{-/-}$ mice for up to 22 weeks post-treatment, while the WT EcN was mostly undetectable across this time period. In contrast, the healthy mice had little to no persistence of the GMO and no observable histopathological changes were observed in the colon and liver of mice receiving the GMO versus the vehicle control (Verdugo-Meza et al., 2024). This suggests that the GMO had an increased ability to colonise an inflamed gut and decreased ability to colonise the healthy gut.

105. The mouse study also showed that the GMO preferentially colonises the large intestine in colitis-prone mice (Verdugo-Meza et al., 2024). This study also demonstrated that in an induced colitis mouse model, mice treated with the GMO had significantly lower clinical scores, lower pathology scores, and lower infiltration of macrophages and neutrophils (drivers of ulcerative colitis) compared to mice treated with WT EcN or the front-line treatment for ulcerative colitis (5-ASA) (Verdugo-Meza et al., 2024).

106. The GMO has also been tested in pigs, which share a more similar anatomy and physiology to humans. Pigs with chemically-induced ulcerative colitis treated with the GMO had increased survival rates compared to the controls (treatment with an empty microcapsule) (Verdugo-Meza et al., 2024). No GM bacteria were detected in the blood, spleen and the mesenteric lymph nodes of the pigs despite the potentially compromised intestinal barrier from the induced disease.

107. There were no adverse reactions to the GMO reported in the mouse or pig studies (Verdugo-Meza et al., 2024).

4.3.4 Clinical trials using other EcN

108. As of October 2025, 16 clinical trials were listed on www.clinicaltrials.gov using EcN for treatment of conditions ranging from colon/gastric cancer; prevention of urinary tract infections; supporting therapy for diabetes; treatment of ulcerative colitis, hay fever, liver disease and irritable bowel syndrome. Outcomes of some of these studies have been reviewed by Falzone *et. al* (Falzone et al., 2024).

109. Most reported clinical trials have used an oral dose of EcN of between 1 x 10^9 and 25 x 10^9 CFU (Kruis et al., 2012; Petersen et al., 2014; Manzhalii et al., 2022; Gurbatri et al., 2024). No adverse events relating to EcN have been reported in these studies (Kruis et al., 2012; Petersen et al., 2014; Manzhalii et al., 2022; Gurbatri et al., 2024).

110. EcN enemas were investigated in a clinical trial for patients with ulcerative colitis, at doses that range from 1 x 10^9 to 4 x 10^9 viable organisms (Matthes et al., 2010). Some adverse events - gastrointestinal and thoracic disorders - were reported but most were deemed to be unrelated to the GMO (Matthes et al., 2010).

111. As mentioned in Section 3.4, the EcN parent strain (commercially known as Mutaflor®) has been used as a probiotic for over 100 years with no reported serious adverse effects in children or adults. There is one report of sepsis (bacterial infection of the blood) by EcN in a pre-term infant with very low birthweight (<1500 g) following probiotic administration (Guenther et al., 2010). However, a larger study involving 405 neonates (newborn up to 28 days after birth; >2000 g) given 10^8 CFU of EcN or a placebo control (empty capsule) showed no obvious difference in adverse events (11.7% vs 8.1%) (Olbertz et al., 2023).

Section 5 The receiving environment

112. The receiving environment forms part of the context for assessing risks associated with dealings with the GMO (OGTR, 2013b). It informs the consideration of potential exposure pathways, including the likelihood of the GMO spreading or persisting outside the site of release.

5.1 Site of administration (Gastrointestinal tract)

113. The primary environment receiving the GM *E. coli* would be the gastrointestinal (GI) tract of the trial participant.

114. In a typical healthy person, whole gut transit time is estimated to be between 10-73 hours, consisting of 2-5 hours for gastric emptying, 2-6 hours to transit the small bowel and 10-59 hours for the colon (Lee et al., 2014). A meta-analysis of the effects of probiotics on intestinal transit time found that they were moderately efficacious in reducing intestinal transit time, but *E.coli* based probiotics were not included (Miller et al., 2016).

115. Antibiotic use impacts the gut microbiome. The effect depends on the class, dosage and duration of the antibiotic treatment as well as factors to do with the individual patient. As well as decreasing the total number of bacteria in the gut, broad spectrum antibiotics can change the balance between bacterial species (Rinninella et al., 2019).

5.2 Presence of related bacterial species in the receiving environment

116. The presence of related bacteria may offer an opportunity for genetic material to transfer between the GMO and other organisms in the receiving environment.

117. The human gut naturally contains a wide range of bacteria as well as archaea, viruses, phages, yeast and fungi. The human colon has been estimated to contain about 1.5 kg of bacteria (Sender et al., 2016). The composition of the human gut bacteria varies between individuals and is affected by diet, lifestyle, medical conditions and treatments, as well as geographical location. The gut microbiota is clearly involved in training the immune system, protecting against colonisation by pathogens, biosynthesising vitamins, energy generation, endocrine function and metabolising drugs and bile salts (Lynch and Pedersen, 2016). There are many other proposed interactions between the microbiome and the host.

118. In healthy adults, 90% of the gut bacteria are Firmicutes and Bacteroidetes. There are smaller amounts of Actinobacteria, Proteobacteria (including *E.coli*), and Verrucomicrobia (Rinninella et al., 2019).

119. Microbiome diversity generally increases with age. The infant gut microbiome is affected by the way they are delivered, antibiotic use and feeding patterns. Babies born vaginally have a gut microbiome similar to that around their mother's birth canal while those delivered by c-section carry bacteria similar to their mother's skin, but these differences reduce over time (Yang et al., 2016) . Additionally, there are

commensal bacteria in healthy human breast milk that are passed from mother to child to help the infant build a healthy microbiome (Murphy et al., 2017) . The gut microbiome of infants may be more easily persistently colonised than adult microbiomes. A study that supplemented breast-fed infants with *Bifidobacterium infantis* EV001 for 28 days found that this bacterium was still the dominant species 60 days later (Frese et al., 2017). Studies of probiotics in adults tend not to show such a dramatic and persistent effect (Zmora et al., 2018). Children are thought to develop a microbiome more similar to adults by around age three (Yang et al., 2016).

120. A large-scale study using human gut genomic data from more than 12,000 individuals, across 45 countries (Europe, North America, Asia and Africa), showed that Enterobacteriaceae was detected in 66% of the individuals (Yin et al., 2025). Enterobacteriaceae in the human gut include several species of Citrobacter, *E. coli*, *Enterobacter cloacae, Enterobacter aerogenes, Enterobacter gergoviae, Klebsiella pneumonia, Klebsiella oxytoca, Morganelle morganii, Pantoea agglomerans, Proteus mirabilis, Serratia marcescens, Serratia plymuthica* (Chung, 2016). The large-scale genomic study determined that *Escherichia, Klebsiella* and *Enterobacter* were the most prevalent genera (Yin et al., 2025). People with ulcerative colitis have an increased population of Escherichia-Shigella (Swirkosz et al., 2023). Therefore, it is likely that participants would have bacteria of similar species present as part of their gut microbiota.

121. Although not documented, the parent EcN is likely to be present in the Australian environment as it is listed in the ARTG (TGA) as a registered complimentary medicine and commercially available for use in Australia. As mentioned in Chapter 1, Section 3.7, *E. coli* can be present in the broader environment (e.g. soil, manure, water). Therefore, it is likely that bacteria of similar species are present in the broader environment.

5.3 Presence of similar genetic material in the environment

122. The balance of a system could be perturbed by the introduction of new genetic material through horizontal gene transfer or through release of GMO into the environment. However, the effect of perturbation would be relatively small if the genetic material was already present in the system and did not confer any selective advantage to an organism that gained this genetic material.

123. The *ttr* operon is derived from *S. enterica* serovar Typhimurium, which is already present in the Australian environment. As previously mentioned, the parent EcN would also be present in the Australian environment. As such, it is likely that organisms in the environment, both in the gut and the broader environment have been exposed to the *ttr* operons in the GMO.

Section 6 Previous authorisations

124. This GMO has not been previously authorised for clinical trials or commercial supply in any region or country. This is a first in human clinical trial.

Chapter 2 Risk assessment

Section 1 Introduction

125. 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 7). 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.

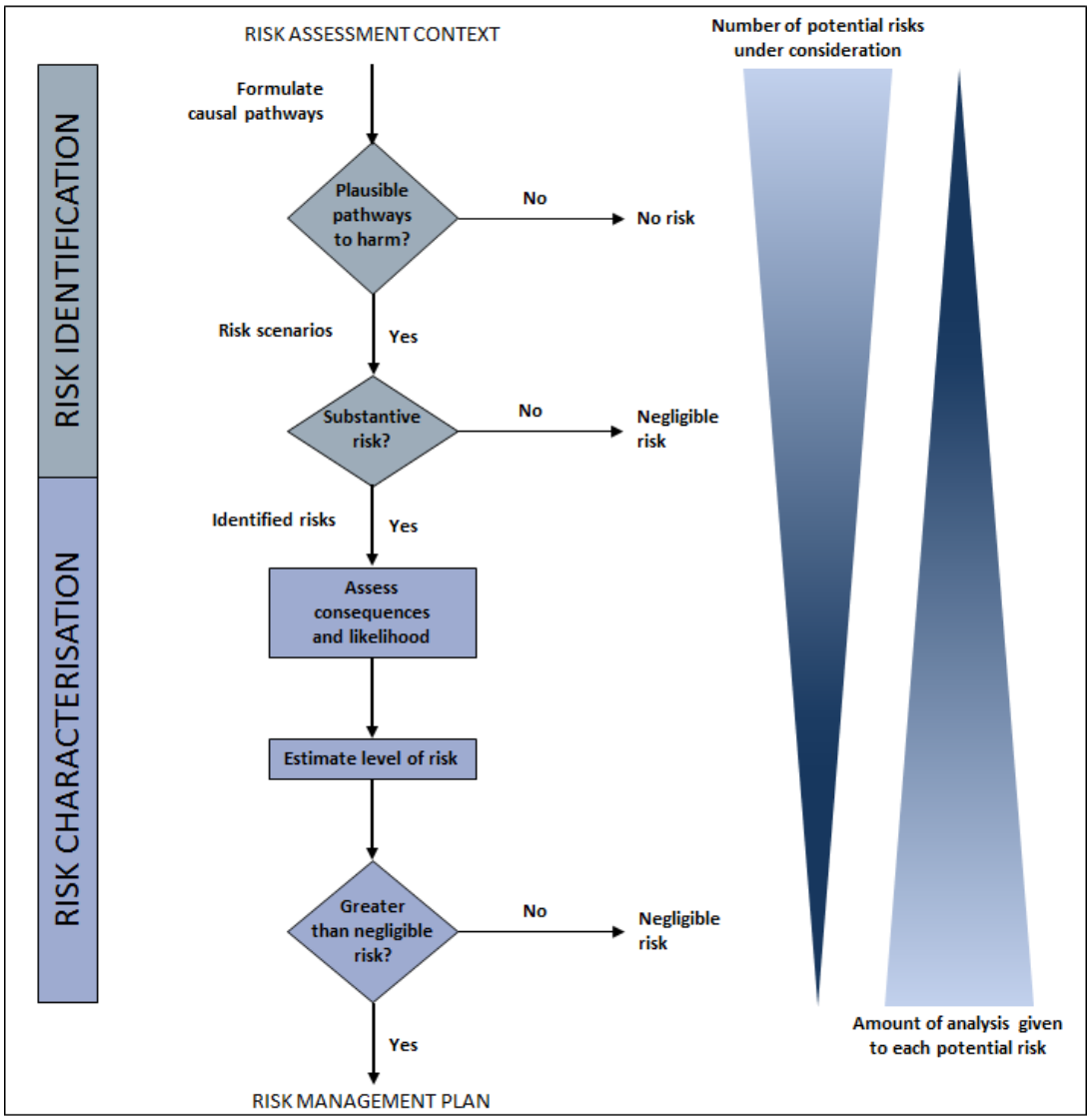

Figure 7: The risk assessment process

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

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

128. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 8), i.e. the risk is considered no greater than negligible.

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

Section 2 Risk identification

130. Postulated risk scenarios are comprised of three components (Figure 8):

i. The source of potential harm (risk source)

ii. A plausible causal linkage to potential harm (causal pathway), and

iii. Potential harm to people or the environment.

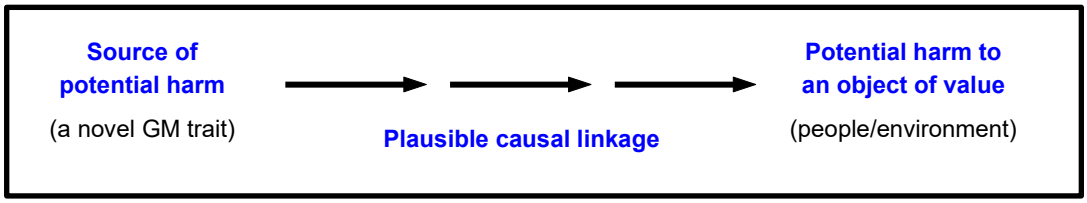

Figure 8: Components of a risk scenario

131. 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

132. The parent organism is the commensal *E.coli* Nissle strain. Details of the properties of the GMO can be found in Chapter 1, Section 4. Transmission of *E. coli* is generally via the faecal-oral route and from contact with faecal material in the environment.

133. Potential sources of harm can be intended novel GM traits associated with one or more of the introduced genetic elements, or unintended effects/traits arising from the use of gene technology. Unintended effects can arise through horizontal gene transfer (HGT), the stable transfer of genetic material from one organism to another without reproduction. All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. A gene transferred through HGT could confer a novel trait to the recipient organism. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism. This pathway is further considered as a potential source of risk.

134. As discussed in Chapter 1, Section 4, the GMO has been modified by the insertion of the *ttr* operon from *Salmonella enterica typhimurium* and deletions in other genes in EcN. These modified genes and their encoded proteins, or effects of deletions, are considered further as a potential source of risk.

2.2 Causal pathway

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

- the proposed dealings, which are conduct experiments (clinical trials), import, transport and disposal of the GMO and possession, supply or use (including storage) in the course of any of these dealings;
- restrictions placed on the import, transport or disposal of the GMO by other regulatory agencies, the States and Territories;
- characteristics of the parent organism;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced or deleted gene(s) and gene product(s) on the properties of the organism;
- potential exposure of other organisms to the introduced gene(s) and gene product(s) from other sources in the environment;
- potential exposure of other organisms to the GMOs in the environment;
- the release environment;
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential);
- environmental stability of the organism (tolerance to temperature, UV irradiation and humidity);
- gene transfer by horizontal gene transfer;
- unauthorised activities; and
- practices before and after administration of the GMO.

136. As discussed in Chapter 1, Section 1.1, the TGA, the trial sponsor, the Investigators and HREC all have roles in ensuring the safety of trial participants under the *Therapeutic Goods Act 1989*, and human clinical trials must be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). Therefore, risk scenarios in the current assessment focus primarily on risks posed to people other than the intended GMO recipient, and to the environment.

137. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harms

138. The following factors are taken into account when postulating relevant risk scenarios for this licence application:

- harm to the health of people or desirable organisms, including disease in humans or animals or adverse immune response to the GMO
- the potential for establishment of the GM *E. coli* in the environment that could cause harm to people or the environment.

2.4 Postulated risk scenarios

139. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 1.

140. In the context of the activities proposed by the applicant and considering both the short and long term, one risk scenario did not give rise to any substantive risk that could be greater than negligible (section 2.4.1; this chapter). Two risk scenarios were identified as posing substantial risks which warranted further assessment (Section 3; this chapter).

Table 1 Summary of risk scenarios from dealings with the GMO

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
1	GMO	Exposure of people (other than the trial participants) or animals (e.g. pets) via aerosols or ingestion during the: (a) Preparation and administration of the GMO (b) Shedding of the GMO (e.g. faeces, diarrhoea, vomit) (c) Import, transport, storage of the GMO (d) Disposal of the GMO ⬇ Colonisation of the GMO in the respiratory tract or gut ⬇ Infection of host cells	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity	No	• The GMO has been modified for increased persistence in gut inflammatory conditions and may not persist as well as WT in a healthy gut. • The dose from accidental exposure during administration or shedding from trial participants would be low. • Only trained personnel wearing PPE would prepare, supervise the administration of, and analyse the GMO. • *E.coli* Nissle strain lacks pathogenic genes. • Import would be in accordance with IATA 3245. • Transport, storage and disposal of the GMO would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.*
2	GMO	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ Transfer of genetic material to or from the GMO	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity	Yes	• See Section 3 for risk characterisation.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
		⬇ Novel bacteria are shed by the participant (e.g. vomit, faeces) ⬇ Exposure of medical staff, carers or pets to novel bacteria ⬇ Colonisation of novel bacteria in the gut ⬇ Infection with novel bacteria			
3	GMO	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ (a) no modification of the GMO or (b) transfer of genetic material to or from the GMO ⬇ GMO and/or novel bacteria are shed by the participant ⬇ GMO and/or novel bacteria enter the environment (e.g. via wastewater) ⬇ GMO and/or novel bacteria establish in the environment ⬇ (a) no further modification or (b) further transfer of genetic material ⬇	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity	Yes	• See Section 3 for risk characterisation.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
		A vulnerable person or animal comes into contact with the GMO and/or novel bacteria ⬇ GMO and/or novel bacteria colonise gut ⬇ Infection with GMO and/or novel bacteria			

2.4.1 Risk scenario 1

Risk source	GMO
Causal pathway	Exposure people (other than the trial participants) or animals (e.g. pets) via aerosols or ingestion during the: (a) Preparation and administration of the GMO (b) Shedding of the GMO (e.g. faeces, diarrhoea, vomit) (c) Import, transport, storage of the GMO (d) Disposal of the GMO ⬇ Colonisation of the GMO in the respiratory tract or gut ⬇ Infection of host cells
Potential harm	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity

Risk source

141. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

142. People (other than the intended trial participants) or animals (e.g. pets) could be directly or indirectly exposed to the GMO in several ways as described below. This exposure could result in colonisation of their gut and alteration of their microbiome leading to ill health (e.g. diarrhoea, vomiting or gut issues) or have genotoxic effects.

Exposure during preparation and administration of the GMO

143. There is potential for exposure of people other than the trial participant to the GMO during the preparation or administration of the GMO via direct contact of persons involved in preparation and administration of the GMO, or via incorrect dispensing of the GMO, which could lead to the wrong person receiving the GMO. This could lead to accidental contact with and/or ingestion of the GMO. The GMO is in microencapsulated form and therefore it is very unlikely that the GMO can form aerosols during the preparation and administration.

144. As discussed in Chapter 1, Section 2.3, the preparation of the GMO would be carried out in clinical trial sites by authorised, experienced, and trained health professionals. The GMO would be self-administered by participants in the presence of trained health professionals, and they would be instructed to wash their hands with soap and water after handling the microencapsulated GMO. All personnel working in settings where healthcare is provided are required to comply with the standard precautions for working with potentially infectious material, as described in the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (2019) and the *Australian Immunisation Handbook.* Compliance with the guidelines, existing work practices and advice to trial participants would minimise the potential exposure of people to the GMOs during preparation and administration of the GMO.

145. The dose received through these pathways would be smaller than that administered during treatment, or in the case of incorrect dispensing, no more than the intended dose. In addition, clinical trials and commercial use of the parent organism have shown no serious adverse effects at doses equivalent to the full dose of the GMO proposed for this trial. Therefore, even if an individual is inadvertently exposed to the GMO, they are unlikely to develop an adverse reaction.

146. As mentioned in Chapter 1, Section 4.2, the GMO has 2 genes deleted (Gene A and Gene B). These modifications would reduce the GMO's ability to survive outside the gut, and potentially its ability to colonise a healthy gut compared to WT, based on mouse studies. Therefore, this may reduce the likelihood of establishment and persistence in a person with a healthy gut.

Exposure due to shedding of the GMO from trial participants

147. It is likely that trial participants would shed the GMO through faeces, diarrhoea, and vomiting. Vomiting is highly unlikely to occur in the healthy trial participants enrolled in this trial. However, vomiting is a symptom of ulcerative colitis, reported to occur in about 25% of patients, although this figure includes patients with severe disease (Newton et al., 2019). Caregivers, healthcare personnel and other people who are in close contact with people treated with the GMO may be inadvertently exposed to the GMO through contact with faeces, diarrhoea or vomit, or after patient use of bathrooms. Pets could also be inadvertently exposed to the GMO from contact with faeces, diarrhoea or vomit. Caregivers, other people or pets exposed to the GMO in this way would only be expected to be exposed to low levels of the GMO. As mentioned in Chapter 1, Section 2.3, trial participants would be instructed to follow good hand hygiene practices to limit surface contamination and prevent hand to mouth transmission.

148. The use of agents that can alter the gut transit time, such as laxatives, anti-diarrhoeal medications, diabetic and weight loss medications, and antibiotics, may increase the likelihood of shedding of the GMO from participants. However, participants who use these agents are excluded from the trial (Chapter 1, Section 2.3.5). If trial participants need antibiotic treatment during the clinical trial, the GMO is likely to be eliminated by commonly used antibiotic treatments and is unlikely to be shed from participants.

149. The GMO is also a probiotic that is intended to reduce the incidence of IBD, which would limit the occurrence of diarrhoea and vomiting.

Exposure during import, transport, and storage of the GMO

150. If the GMO was dispersed during import, transport and storage, this could result in exposure to people or animals in the area via contact with materials or surfaces contaminated with the GMO and subsequent hand to mouth transmission.

151. The GMO would be imported, stored, and transported according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSDs) (Chapter 1, Section 2.3.2). Additionally, the GMO is supplied in an encapsulated form (beads), so unless animals were able to access and consume the beads, or the beads were damaged allowing direct access to the GMO, contact with the

GMO is unlikely. In addition, biological samples that may contain GMO would also be treated in accordance with the TSDs. These practices would lower the likelihood of unintended dispersal of the GMOs.

152. As mentioned in Chapter 1, Section 2.3.8, decontamination and disinfection measures appropriate for the GMO would be carried out after administration of the GMO or if dispersed during the supply of the GMO.

153. The import, transport and storage procedures discussed above would mitigate exposure to the GMO during these dealings.

Exposure during disposal of the GMO

154. Individuals may be inadvertently exposed to GMOs while disposing of used, expired, or unused vials of the GMO. The two locations where this is most likely to occur are at:

- locations where stocks of the GMO are stored,
- locations where the GMO is administered.

155. As discussed in Chapter 1, Section 2.3.8, unused and expired blister packs, vials or bottles of the GMO, as well as waste contaminated with the GMO would be treated as clinical/medical waste and disposed of in accordance with the waste disposal methods approved by the Environmental Protection Agency or Health Department in Queensland (The Queensland Government, 2025). Adherence to these procedures would reduce the likelihood of accidental exposure of people or animals to the GMO.

156. Taken together, the disposal and decontamination procedures discussed above would minimise likelihood of exposure that could be associated with conducting these dealings with the GMO.

Potential harm

157. If people or animals are exposed to the GMOs, the GMO could establish in the gut and result in an altered microbiome. An altered microbiome could lead to persistence and long-term exposure of the GMO, which could potentially result in ill health (e.g. diarrhoea, vomiting or gut issues). As noted in Chapter 1, Section 3.4, the parent EcN could potentially have genotoxic effects due to the expression of colibactin and if the GMO established in the gut, this expression could persist. However, it is not expected that the genetic modifications would result in increased likelihood of genotoxic effects compared to the parental EcN strain. Additionally, exposure is unlikely to have negative effects of ill-health because:

- the parent organism used to generate the GMO has a long history of safe use and the modification carried out is unlikely to increase to capacity of the GMO to cause harm;
- pre-clinical studies with the GMO indicated that it did not cause severe disease and it was shown to alleviate the symptoms of IBD;
- the GMO is likely to be less effective in colonising a healthy gut than the EcN parental strain based on mouse studies;
- the GMO is less stable in the broader environment compared to the parent organism;
- although there is some literature regarding the possibility of genotoxicity associated with colibactin, which is produced the EcN parental strain, there is currently no publicly available information showing that EcN causes cancer in humans despite the probiotic being in use for over 100 years;
- most people recover from *E. coli* infections on their own with rest and uptake of fluids to prevent dehydration;

- effective antibiotic treatments are available if needed.

158. As mentioned in Chapter 1, Section 3.4, the parent organism used to generate the GMO has been used in various animals without reports of any adverse events. In addition, pre-clinical studies with the GMO did not report any adverse effects (Chapter 1, Section 4.3.3). The modifications carried out in the GMO are unlikely to increase the capacity of the GMO to cause harm in comparison to the parent organism. Therefore, the potential harm to animals is highly unlikely.

159. The use of probiotics (*Lacticaseibacillus* spp.*, Bifidobacteria* or *Bacillus subtilis*) have been linked to infections, including sepsis, in immunocompromised individuals or those with pre-existing health conditions (Redman et al., 2014; Katkowska et al., 2021). As mentioned in Chapter 1, Section 4.3.4, there is only one report of sepsis that is attributed to EcN, this was in an infant with very low birthweight. However, it is important to note that these infections have been attributed to the consumption of probiotics by these individuals and not via transmission of the probiotics from another person. The potential exposure via transmission is likely to be at a much lower dose that the recommended dosage. Although there is limited information available regarding the possibility of *E. coli* strains resulting in sepsis in immunocompromised individuals, it is unlikely that they would be exposed to sufficient GMO to cause serious harm. However, this is an area of some uncertainty.

Conclusion

160. The potential for an unintentional exposure of people and animals to the GMO to cause harm via the alteration of the gut microbiome or genotoxicity in humans and animals is not identified as a risk that could be greater than negligible. Therefore, this risk scenario does not warrant further detailed assessment.

Section 3 Risk characterisation

161. Three risk scenarios were postulated and evaluated, as summarised in Table 1. Risk scenarios 2 and 3 were identified as posing substantive risks which warrant further assessment. This section provides more detail on the characterisation of these risks.

162. The risks of harm that were considered substantive are the risks:

- to people (other than the trial participants) and animals through ill health caused by infection with novel bacteria as a result of horizontal gene transfer; and
- from the spread and persistence of the GMO or novel bacteria.

163. Therefore, a risk characterisation was performed for each of these risk scenarios. Risk characterisation involves a likelihood assessment, a consequence assessment, a risk estimate, and a decision on whether risk treatment is required. In summary, a likelihood assessment determines the chance that harm may occur, ranging from highly unlikely to highly likely. The consequence assessment determines the degree of potential harm, ranging from marginal to major. The final risk estimate considers both the likelihood and consequence assessments to estimate the level of risk, ranging from negligible to high. See the Risk Analysis Framework for further information about the OGTR's approach to conducting risk analysis.

3.1 Risk scenario 2

Risk source	GMO
Causal pathway	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ Transfer of genetic material to or from the GMO ⬇ Novel bacteria are shed by the participant (e.g. vomit, faeces) ⬇ Exposure of medical staff, carers or pets to novel bacteria ⬇ Colonisation of the novel bacteria in the gut ⬇ Infection with novel bacteria
Potential harm	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity

3.1.1 Risk source

164. The source of potential harm for this postulated risk scenario is the GMO.

3.1.2 Causal Pathway and likelihood assessment

165. Following administration, it is expected that the GMO will colonise the trial participant's gut. This could result in the establishment of the GMO in the gut and through transfer of genetic material between the GMO and other bacteria colonising the participant's gut, could result in complete or partial reversion of the GMO to the WT phenotype or in the generation of other novel bacteria in the gut that have acquired the *ttr* operon (discussed below). The novel bacteria resulting from HGT could then be shed by the participant and transmission could occur via the pathways discussed in Risk scenario 1.

166. The GMO has been modified by the insertion of the *ttr* operon and the deletion of 2 genes from the parent, EcN (Chapter 1, Sections 4.1 and 4.2). The insertion of the *ttr* operon increases the ability of the GMO to persist in inflammatory conditions, which are common in ulcerative colitis. The deletion of Gene A reduces the GMO's ability to survive outside the gut. The deletion of Gene B (as a result of the insertion of the *ttr* operon from *S. enterica*) may reduce its ability to colonise a healthy gut, based on information from mouse studies, although there is some uncertainty about this effect.

167. A large-scale study examining human gut genomic data from over 12,000 people across 45 countries showed that 66% of gut microflora were Enterobacteriaceae and that *Escherichia, Klebsiella* and *Enterobacter* were the most prevalent genera (Chapter 1, Section 5.3). The presence of such a range of compatible gut bacteria indicates that there is potential for HGT between the GMO and other compatible bacteria.

Changes to the GMO as a result of HGT resulting in novel bacteria

168. There are several potential changes that could occur in the GMO through HGT (Chapter 1, Section 3.5.1). The GMO could lose the *ttr* operon and gain Gene A or Gene B or both. However, multiple recombination events would be required for the GMO to reacquire both genes and lose the *ttr* operon (or combinations of these). If the GMO:

- loses the *ttr* operon, it would have a reduced ability to colonise an inflamed gut compared to the GMO. However, it would retain the other characteristics of the GMO.
- reacquires Gene A, it would regain its ability to survive in the environment.
- reacquires Gene B, it could potentially restore its ability to colonise a healthy gut (noting that the acquisition of Gene B via homologous recombination may result in the loss of the *ttr* operon).
- reacquires both Gene A and Gene B, it may have similar characteristics to the parent EcN strain (similar ability to survive in the environment and potentially similar ability to colonise a healthy gut), but with an increased ability to colonise an inflamed gut. However, the acquisition of the *ttr* operon by the parent EcN that is currently used as a probiotic can already occur naturally from other bacteria in the gut that contain the *ttr* operon (e.g. from *S. enterica*).
- reacquires both deleted genes and loses the *ttr* operon, it would have similar characteristics to the parent EcN strain.

169. The parent EcN is not known to be pathogenic, and as discussed previously, the modifications to produce the GMO are unlikely to affect its pathogenicity. It is unlikely that the changes to the GMO due to HGT would result in harm to people or the environment.

Generation of novel bacteria via HGT

170. Similarly, there are several potential changes that could occur in other bacteria that are present in the gut of participants through HGT. However, the only event that could potentially result in an increased harm from novel bacteria is the transfer of the *ttr* operon from the GMO.

171. The *ttr* operon has been inserted into the chromosomal DNA of the GMO (Chapter 1, Section 4.1). There is potential for the *ttr* operon to be transferred to other pathogenic or non-pathogenic bacteria present in the gut of the participants, resulting in novel bacteria that could have an increased ability to colonise an inflamed gut.

172. If non-pathogenic bacteria acquire the *ttr* operon, it is unlikely that it could cause harm. However, if pathogenic bacteria acquire the *ttr* operon, this may result in a novel strain of pathogenic bacteria with an increased ability to persist in inflammatory conditions in the gut. However, the *ttr* operon is already present in the environment as it is isolated from *S. enterica*.

173. Any bacteria that receive the *ttr* operon as a result of HGT from the GMO can then be shed by the participants and transmission of the novel bacteria can occur via the pathways mentioned in Risk scenario 1.

174. If gut bacteria transfer either or both deleted genes (Genes A and B) to the GMO via homologous recombination, the donor bacteria would have a reduced ability to survive in the environment and/or could theoretically have reduced ability to colonise a healthy gut.

Mechanisms for reversion and HGT

175. The main mechanisms for HGT in bacteria are transformation, transduction, transposition and conjugation (Chapter 1, Section 3.5.1). Conjugation is unlikely because the modifications are on the chromosomes of the GMO, not on the plasmid.

176. Transformation involves the uptake of released DNA fragments from the environment by competent bacteria. In order to be competent, bacteria must have specific genes to allow them to take in DNA and receive the correct environmental signals (Blokesch, 2016). In this case, DNA fragments containing *ttr* operon could come from dead GMO. Free DNA is unlikely to survive for long in the participant's gut due to the presence of deoxyribonucleases (DNases), enzymes that degrade DNA. People with severe ulcerative colitis still show DNase activity in serum (63 ± 19%) although it is significantly less ($p < 0.001$) than that of healthy people (92 ± 11%) (Malíčková et al., 2011), so it is possible that it may survive for a longer period in an inflamed gut.

177. The *ttr* operon from *S. enterica* is inserted into the GMO in the region where Gene B is located (Chapter 1, Section 4.1). For transduction to occur, a bacteriophage must infect the GMO and then carry the *ttr* operon to a second bacteria. The bacteriophage would then need to integrate into the second bacteria's genome as a prophage. If transduction occurs, the GMO could regain Gene B from other gut bacteria, and the other bacteria will gain the *ttr* operon but lose Gene B via homologous recombination, potentially resulting in the GMO having the ability to colonise a healthy gut similar to that of the WT. Alternatively, the *ttr* operon could be inserted in other regions in the *E. coli* and bacteriophage genome that have specific attachment sites. In the same way, the GMO could reacquire Gene A from a second bacteria.

178. Transduction does not occur at high frequency and bacteria often have defence mechanisms against bacteriophages because integration into the chromosome has the potential to kill the bacteria if it occurs in the wrong location. It is generally accepted that HGT involving chromosomes is less efficient and less common than HGT involving plasmids (Moura de Sousa et al., 2023; Wang et al., 2023).

179. EcN has been shown to be resistant to infection by T4 bacteriophage, which is a species of bacteriophage that infect *E. coli*. Resistance to T4 bacteriophage infection is due to the K5 polysaccharide capsule that forms the outer layer of EcN and the type of lipopolysaccharide expressed by EcN (Soundararajan et al., 2019). EcN has also been shown to be resistant to infection with lambda bacteriophage (isolated from non-pathogenic K-12 *E. coli*) and Shiga toxin bacteriophages (found in pathogenic *E. coli*). This resistance was attributed to the expression of a phage repressor gene (*pr*) (Bury et al., 2018).

180. In contrast, another study has shown that EcN is susceptible to infection with a Shiga toxin bacteriophage (Stx2) isolated from a pathogenic *E. coli* and could result in EcN expressing Shiga toxin (Pradhan, 2020). However, for this to occur, both bacteria would need to be present in the gut. The applicant has indicated that participants will be excluded from the trial if they have had a diagnosis of any non-inflammatory bowel disease-related diarrhoeal illness such as bacterial or parasitic infections within the last 3 months or have received faecal microbiota transplantation (FMT) or other faecal-derived preparations within 6 months prior to randomisation. Therefore, the likelihood of the presence of pathogenic *E. coli* in the participant's gut is minimised. It is possible that a trial participant could acquire an infection with pathogenic *E. coli* during the clinical trial. However, EcN has been shown to directly inhibit the growth of various pathogenic bacteria, likely by competing with harmful bacteria for resources as discussed in Chapter 1, Section 3.4. Therefore, the likelihood of infection of the GMO with Shiga toxin bacteriophages is also minimised.

181. The bacteriophage attachment sites used to insert the *ttr* operon into the GMO have been deleted and this is likely to result in increased stability of the insert by reducing the potential for transduction to occur.

182. Transposition of the *ttr* operon could occur via the transfer of the *ttr* operon from the GMO to other bacteria via transposable elements or transposons, as discussed in Chapter 1, Section 3.5.1. In the same way, Gene A and B could be transferred from other bacteria to the GMO. In general, transposition is an infrequent event, probably due to its capacity to cause deleterious effects in the host. Usually, a transposon is translocated onto a plasmid upon conjugation. This may be followed by

the integration of the transposon into the chromosome. For many transposons, however, plasmids rather than the bacterial chromosome appear to be the preferred target (Craig, 2014). The estimated rate of transposition for *E. coli* is reported to be between 3.5 x 10^{-4} and 1.15 x 10^{-5} per genome per generation (Sousa et al., 2013; Lee et al., 2016).

183. The *ttr* operon has also been isolated in a novel strain of *E. coli* and is present in 1% of the *E. coli* genomes in the NCBI database (Chapter 1, Section 5.2). Therefore, it is already present in some *E. coli* found in the microbiota of humans and so HGT of the *ttr* operon could occur in the gut from these non-GM *E. coli*, as well as from other bacteria containing the *ttr* operon.

184. As mentioned in paragraph 179, the exclusion criteria for the clinical trial would also reduce the likelihood of the GMO transferring the *ttr* operon to a pathogenic bacterium in the participant's gut.

185. If novel bacteria are shed through faeces, diarrhoea or vomit, they could potentially persist on surfaces for weeks to a year if not correctly decontaminated with suitable disinfectants (see Chapter 1, Section 3.7). As mentioned in Chapter 1, Section 2.3, trial participants would be instructed to follow good hand hygiene practices to limit surface contamination.

186. Based on the information described above and the limits and controls described in Risk scenario 1, the likelihood of harm caused by a novel bacteria resulting from HGT is assessed as **highly unlikely** (harm may occur only in very rare circumstances).

3.1.3 Consequence assessment

187. If the GMO completely reverts to the WT parent organism via pathways described above, it is highly unlikely to cause any harm, even in immunocompromised individuals, as discussed in Risk scenario 1. Therefore, the consequence of exposure to other people to the reverted WT parent organism would be **marginal** (minimal or no increase in illness/injury to people or desirable components of the environment).

188. If the GMO regains Gene A and/or Gene B, it is likely to survive better in the environment and/or potentially regain its ability to colonise a healthy gut. However, the GMO is not pathogenic and although it could potentially persist better in inflammatory conditions, the novel bacteria is not likely to be more pathogenic than the GMO and is unlikely to cause any harm to people or the environment. Therefore, the consequence of exposure to other people to novel bacteria that has regained Gene A and Gene B would be **marginal.**

189. If other non-pathogenic or pathogenic bacteria in the gut were to acquire the *ttr* operon, it may give them an advantage to persist in an inflammatory environment. However, the acquisition of the *ttr* operon by other bacteria in the gut could result in the loss of Gene B via homologous recombination. The deletion of Gene B has been associated with a reduced capacity to colonise the healthy gut compared to WT in mouse studies although this is yet to be determined in humans. If novel bacteria lose Gene B, it may have a reduced ability to persist in a healthy gut or would be similar to the parent bacteria in this regard, thus would be highly unlikely to cause greater harm than the WT bacteria.

190. Exposure of people experiencing inflammatory bowel disease or gut inflammation from any other cause to novel non-pathogenic bacteria containing the *ttr* operon is unlikely to cause any greater harm as it would only increase the persistence of novel bacteria that do not cause disease. Hence, the consequence of exposure of people other than the trial participants to novel non-pathogenic bacteria receiving the *ttr* operon is **marginal**.

191. Exposure to novel pathogenic bacteria may cause bacterial infection (e.g. diarrhoea, stomach cramps, vomiting). Most bacterial infections can be treated with antibiotics if needed, but in some cases of severe bacterial infection, hospitalisation may be required. Considering this, the consequence of exposure of people other than the trial participants to novel pathogenic bacteria

containing the *ttr* operon ranges from **minor** (*minor increase in illness/injury to people that is readily treatable*) if the infection can be treated with antibiotics to **intermediate** (*significant increase in illness/injury to people that requires specialised treatment*) if hospitalisation is required.

3.1.4 Risk estimate

192. The risk estimate is based on a combination of the likelihood and consequence assessments, using the Risk Estimate Matrix, as described in the Regulator's Risk Analysis Framework (OGTR, 2013a).

193. The likelihood of novel bacteria causing harm is considered **highly unlikely.** The potential consequence to the health of people or to the environment is considered **marginal** to **intermediate**. The overall risk is therefore estimated to be **negligible** (*risk is of no discernible concern and there is no present need to invoke actions for mitigation*) to **low** (*risk is of minimal concern but may invoke actions for mitigation beyond standard practice*).

3.2 Risk scenario 3

Risk source	GMO
Causal pathway	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ (a) no modification of the GMO or (b) transfer of genetic material to or from the GMO ⬇ GMO and/or novel bacteria are shed by the participant ⬇ GMO and/or novel bacteria enter the environment (e.g. via wastewater) ⬇ GMO and/or novel bacteria establish in the environment ⬇ (a) no further modification or (b) further transfer of genetic material ⬇ A vulnerable person or animal comes into contact with the GMO and/or novel bacteria ⬇ GMO and/or novel bacteria colonise gut ⬇ Infection with GMO and/or novel bacteria
Potential harm	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity

3.2.1 Risk source

194. The source of potential harm for this postulated risk scenario is the GMO.

3.2.2 Causal Pathway and likelihood assessment

195. The GMO and/or novel bacteria could be shed from trial participants as described in Risk scenarios 1 and 2, including shedding in faeces, diarrhoea or vomit, which could result in the GMO being released into the environment. This could lead to the establishment of the GMO or novel bacteria in the environment, which could result in exposure of people and animals (including marine or aquatic animals) to the GMO or novel bacteria.

196. The risk from accidental dispersal during the preparation and administration of the GMO and during the import, transport, storage and disposal of the GMO, as well as risks associated with the shedding of the GMO (e.g. faeces, diarrhoea, vomit) within the clinical trial site and at home have been addressed in Risk scenario 1. Risk scenario 2 considered risks from the direct exposure to novel bacteria. This risk scenario will discuss the potential of the GMO or novel bacteria entering and persisting in the environment as a result of the excretion of human faecal waste.

Wastewater treatment of human faecal waste

197. Section 3.6 in Chapter 1 discusses the effects of wastewater treatment on bacterial populations and the use of sewage as biosolids in agriculture in detail. The summary below includes information relevant to this risk scenario.

198. In urban areas, most wastewater is processed at centralised wastewater treatment plants (WWTPs). Processes at WWTPs vary, with either 2 or 3 stages of treatment after which most wastewater is returned to the ocean, a lake, or a river.

199. Efficacy of wastewater treatment for removal of bacteria varies considerably. One UK study showed that the majority of faecal bacteria were removed at the secondary or tertiary stages of decontamination (Kay et al., 2008), with an overall reduction in bacteria of up to 3000-fold.

200. An analysis of 4 wastewater treatments plants across Australia found that an average of 126 different genera of bacteria were present (Ahmed et al., 2017). The 10 most abundant genera were *Pseudomonas, Arcobacter, Bacteroides, Paludibacterium, Conchiformibius, Flavobacterium, Polynucleobacter, Acinetobacter, Parabacteroides,* and *Cloacibacterium.* A study of 4 WWTPs in Queensland found that sometimes human pathogenic *E. coli* could survive tertiary treatment and reach the environment (Anastasi et al., 2010). Determining the number of *E. coli* in the environment that came from waste water is complicated by birds and other animals carrying similar *E.coli* to humans (Anastasi et al., 2012).

201. Other systems are used for waste disposal such as septic systems, aerated wastewater treatment system and dry composting toilets. Participants could also use non-standard toilets during activities such as camping. Such systems are generally less effective at killing bacteria than wastewater treatment plants.

202. The reduction in bacterial load and dilution of waste in larger volumes of wastewater that would occur in the wastewater treatment process mean that bacterial concentrations in areas where wastewater is released are likely to be very low. Competition and dilution still occur in other waste disposal systems but to a lower extent, thus the bacterial concentrations at release sites from these methods may be higher.

203. The ideal temperature for most enterobacteriaceae is 37°C, so they are not very well adapted to cold temperatures, and do not proliferate well in waterways or the ocean (Bogosian et al., 1998). UV radiation from the sun also kills enterobacteriaceae. Therefore, it is likely that the amount of any novel enterobacteriaceae remaining in these environments would be low.

204. If a person, a land-based animal or a bird were to ingest water directly from an environment where the effluent is released, the amount of GMO or novel bacteria is expected to be very low and is unlikely to be at a sufficient concentration to colonise the gut and/or to cause illness. Fish and

other aquatic animals generally cannot be colonised by human gut bacteria due to their lower body temperatures (Del Rio-Rodriguez et al., 1997).

205. The modifications carried out on the GMO would not increase the ability of bacteria to survive the wastewater treatment process or its ability to survive in the environment. The GMO or any novel bacteria containing the *ttr* operon is likely to be outcompeted by wild-type bacteria that are not paying the metabolic cost to maintain a gene (*ttr* operon) that does not confer any advantage in the aquatic environment. In addition, a gene has been deleted from the GMO that reduces its survivability in the environment as is unable to produce an essential factor and would need rely on an external source. If any novel bacteria containing the *ttr* operon, but without the gene deletions were released, they would not have reduced ability to survive in the environment, but they would also not have any survival advantage.

206. Biosolids from sewerage plants may be used for agricultural purposes, although in Queensland, they must meet the requirements for <100 most probable number (MPN) of *E. coli* per gram of dry weight (Chapter 1, Section 3.6). Therefore, it is possible that the GMO or novel bacteria could be present in the environment as biosolids. However, as the numbers of participants is low (36 including placebo controls) and the presence of other bacteria, environmental factors and modifications to the GMO that reduces its survivability in the environment and any novel bacteria would not have a survival advantage in the environment, it is unlikely that the GMO or novel bacteria could outcompete other bacteria present in the environment and persist.

Limited number of trial participants

207. The applicant has proposed that there would be 36 participants in the study, with 9 of those participants receiving a placebo. These further limits the amount of GMO or novel bacteria that could be shed into the environment.

208. Based on the information described above and the limits and controls described in Risk scenario 1, the likelihood of the GMO or novel bacteria to persist in the environment and cause harm to people and the environment is **highly unlikely** (harm may occur only in very rare circumstances).

3.2.3 Consequence assessment

209. The consequence assessment would be the same as considered in Risk scenarios 1 and 2, which is **marginal** to **intermediate**.

3.2.4 Risk estimate

210. The risk estimate is based on a combination of the likelihood and consequence assessments, using the Risk Estimate Matrix, as described in the Regulator's Risk Analysis Framework (OGTR, 2013a).

211. The likelihood of the GMO or novel bacteria to be released into the environment and result in disease in people or animals is considered **highly unlikely.** The potential consequence to the health of people or to the environment is considered **marginal** to **intermediate.** The overall risk is therefore estimated to be **negligible** (*risk is of no discernible concern and there is no present need to invoke actions for mitigation*) to **low** (*risk is of minimal concern but may invoke actions for mitigation beyond standard practice*).

Section 4 Uncertainty

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

213. 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 for estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

214. As this is a first in human clinical trial, there is no available clinical biodistribution and shedding data for this GMO. Pre-clinical data using the GMO and clinical data from similar GMOs have been considered in this assessment.

215. Although the GMO is likely to produce a colibactin (a genotoxin), there is uncertainties on whether the GMO can cause cancer. However, the parent organism used to generate the GMO has been safely used as a probiotic for over 100 years. Additionally, there is no information to suggest that the genetic modification proposed for this clinical trial would impact the production of colibactin compared to the parental EcN strain. However, this remains an area of some uncertainty.

216. There is information from mouse studies indicating that the deletion of Gene B reduces the ability of the GM *E. coli* to colonise a healthy gut compared to WT, based on mouse studies. However, there is uncertainty about whether the deletion of Gene B will result in a reduced ability for the GMO to colonise a healthy human gut.

217. The uncertainties outlined above have been accommodated by taking a conservative approach to the risk analysis.

218. Post release review (Chapter 3, Section 4) will be used to address uncertainty regarding future changes to knowledge about the GMO. This is typically used for commercial releases of GMOs, which generally do not have fixed duration.

Section 5 Risk evaluation

219. 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.

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

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

221. Three risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether people and animals can be exposed to the GMO while conducting the dealings and whether there is a potential for HGT of the GMO with other bacteria. The potential for the GMO to be released into the environment and its effects were also considered.

222. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance in the risk assessment process.

223. In the context of the range of measures already in place, including the operating guidelines and requirements of the other regulatory agencies, and considering both the short and long term, one of these scenarios was not identified as representing a substantive risk requiring further assessment. The principal reasons for this include:

- the GMO is unlikely to be shed from recipients except in faeces and vomit;

- the likelihood of accidental exposure to the GMO in people not being treated or animals would be minimised due to well-established import, transport, storage and disposal procedures;
- limited ability and opportunity for the genetic modification to be transferred by horizontal gene transfer mechanisms; and
- survival and persistence of the small amount of GMO in the Australian aquatic and terrestrial environment is highly unlikely.

224. Risk scenarios 2 and 3 describe pathways where HGT could potentially result in novel bacteria that could cause adverse health effects. These risk scenarios were identified as substantive risks, so further assessment was required.

225. The likelihood and consequences of the substantive risks were characterised (Chapter 2, Section 3), and the level of risk estimated using the Risk Estimate Matrix, as described in the Regulator's Risk Analysis Framework (OGTR, 2013a). Following risk characterisation, the risks described in Risk scenario 2 and Risk Scenario 3 were estimated as posing a **negligible to low** risk to human health and safety and the environment.

226. The Risk Analysis Framework describes **negligible** risks as risks of no discernible concern where there is no present need to invoke actions for mitigation and **low** risks as risks of minimal concern that may invoke actions for mitigation beyond standard practice. Measures to mitigate the identified risks are proposed in Chapter 3, Section 2.

227. Determination of whether a risk is considered to be significant, and therefore whether a longer consultation period is required for the consultation RARMP, are made on a case-by-case basis. As the proposed mitigation measures can manage the risk to people and the environment, the Regulator considered 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

228. 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.

229. 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.

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

231. 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. 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

232. The risk assessment of Risk scenarios 2 and 3 listed in Chapter 2 concluded that there are negligible to low risks to people and the environment from the proposed clinical trial with the GMO. The risk stems from the potential acquisition of the *ttr* operon by pathogenic bacteria from the GMO and release of these pathogenic bacteria into the environment.

233. One key route of exposure to the GMO is via the faecal oral transfer, which could occur through poor hand hygiene, during sexual activity with participants receiving the GMO, or through inadequate cleanup of vomit and faeces/diarrhoea.

234. To manage transmission through poor hand hygiene, a condition included in the licence requires the licence holder to provide the participants with instructions of proper hand hygiene and sample collection procedures, and to obtain written agreement from the participants that they can comply with these behavioural requirements.

235. To manage transfer during sexual activity, the applicant has proposed measures to ensure double barrier contraceptives and abstinence from unprotected anal sex for the duration of the trial to manage any sexual activity that may result in faecal oral route of exposure. However, to capture all forms of sexual activity, a condition is included in the licence to ensure that participants agree to use effective methods to prevent faecal oral transmission when engaging in sexual activities that may result in the transmission of the GMO.

236. To minimise exposure of other people to the GMO during the cleanup of vomit or faeces/diarrhoea, the applicant has proposed appropriate decontamination procedures if these situations occur in the clinical trial. However, this could also occur in the participants' homes. Therefore, to further minimise exposure of other people while cleaning up vomit or faeces/diarrhea in non-clinical settings, a condition is included in the licence requiring the licence holder to provide

instructions to participants to decontaminate any vomit or faeces/diarrhoea using decontamination agents that are effective against the GMO.

237. The risk assessment for Risk Scenario 1 concluded that there are negligible risks to people and the environment from the proposed clinical trial with the GMO. This risk scenario was considered in the context of the scale of the proposed clinical trial (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), the proposed receiving environment (Chapter 1, Section 5), and considering both the short- and long-term risks. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks.

238. Limits and controls proposed by the applicant and other general risk management measures are discussed below

Section 3 General risk management

239. 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 to low. Therefore, to maintain the risk context, licence conditions have been proposed to limit the number of trial participants, limit the location of the trial to hospitals and clinical trial sites, limit the duration of the trial, as well as a range of controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the licence.

3.1 Limits and controls on the clinical trial

240. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by Melius. Many of these are discussed in the 3 risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 Consideration of limits and controls proposed by Melius

241. The proposed clinical trial would involve a maximum of 36 participants within Australia, and dealings related to storage, preparation and administration of the GMOs would take place in medical facilities such as a hospital or clinical trial facilities in Brisbane. Activities that would occur outside of medical facilities include transport, storage and disposal of the GMOs. The applicant has proposed to complete dealings with the GMO within 5 years of commencement. A licence condition limits the period when the GMO may be administered under the licence to 5 years from the date of issue of the licence. Other conditions maintaining the risk context and proposed limits of the trial such as a maximum of 36 trial participants and requirements for dealings related to preparation and administration of the GMO to be conducted at a clinical trial site are included in the licence. Licence conditions do not limit the trial to be carried out in Brisbane as any hospitals or clinical trial facilities in Australia would be appropriate to carry out this clinical trial.

242. The applicant advised that import and transport of the GMO and waste containing the GMO would be in accordance with IATA UN 3245 and the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, respectively. IATA UN 3373 would also meet the requirements for the import of the GMO. These are standard protocols for the handling and minimising exposure to the GMOs. Once at the clinical trial site, access to the GMO would be restricted to appropriately trained personnel. These proposed transport conditions are suitable for the GMO. Therefore, the licence details the minimum requirements for packaging and labelling the GMO and waste contaminated with the GMO, for transport and storage within a clinical trial site, as well as transport of the samples that may contain GMO for analysis or waste disposal. Additionally, conditions would require the import of the GMO should be carried out in accordance with IATA UN 3245 or UN 3373. These measures would limit the exposure of people and the environment to the GMOs.

243. Proposed inclusion and exclusion criteria for trial participants are listed in Chapter 1, Section 2.3.5. The inclusion and exclusion criteria for trial participants would be subject to approval by a HREC, who would consider the safety of the individuals involved in the trial.

244. The relevant inclusion criteria proposed by the applicant that would limit transmission that are considered here include that the trial participants must:

- agree to use effective double barrier contraceptives and abstain from unprotected anal sex for the duration of the trial;
- be of non-childbearing potential (women) or agree to use barrier contraceptive.

245. The relevant exclusion criteria proposed by the applicant that would limit shedding and/or HGT include:

- having a diagnosis of any non-Inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections) within three months prior to randomisation;
- use of probiotics within 2 weeks prior to randomisation;
- use of agents (e.g. laxatives, anti-diarrhoeal medications and diabetic or weight loss medications) that may alter gut transit time that could lead to more shedding;
- receiving faecal microbiota transplantation (FMT) or other faecal-derived preparation within 6 months prior to randomisation;
- use of antibiotics.

246. As stated in the risk scenarios, the GMO can potentially be shed in faeces and vomit. The applicant has proposed to give participants instructions for collecting samples at home where applicable. The applicant would provide sufficient containers and sealable plastic bags to ensure transport between the participant's home, and the site of analysis meet the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.* A condition requiring the licence holder to obtain written agreement that the trial participants would comply with the written instructions regarding sample and storage procedures is also included in the licence. The licence also requires the licence holder to provide the written instructions to the Regulator, if requested. Conditions to limit the faecal oral route of transmission are included in the licence, particularly in relation to provision of information about hand hygiene and agreement of participants to behavioural conditions related to sexual activity, are discussed in Section 2 of this chapter.

247. Participants with non-inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections); the use of agents (e.g. laxatives, anti-diarrhoeal medications and diabetic or weight loss medications) that may alter gut transit time; and antibiotics that may cause antibiotics-associated diarrhea could affect the potential shedding of the GMO. Therefore, a condition to exclude participants that have a diagnosis of non-inflammatory bowel disease within 3 months; participants that are using agents that may alter gut transit time; and participants that are currently using antibiotics have been included in the licence. As discussed in Chapter 2, Section 2.4.1, trial participants may require antibiotic treatment during the clinical trial. If this occurs, the GMO is likely to be eliminated by commonly used antibiotic treatments and is unlikely to be shed from participants. Therefore, the licence does not exclude trial participants from receiving antibiotic treatment while taking the GMO.

248. The presence of other bacteria in the gut may affect HGT as discussed in Risk scenario 2. Other bacteria could be from pre-existing bacteria in the gut or from introduced bacteria (e.g. use of probiotics). Participants that potentially have diarrhoeal illness due to bacterial infections have already been excluded as mentioned in paragraph 245. This exclusion would reduce the likelihood that the GMO would encounter pathogenic bacteria for HGT to occur and result in a novel

pathogenic bacterium. In general, the persistence of probiotics in the gut is low (around 2 weeks) and hence continuous dosage is required to maintain their population in the gut. However, probiotics are not pathogenic, and it is unlikely that transfer of genetic material from the GMO via HGT would result in harm. Therefore, the exclusion of people that have used probiotics within the last 2 weeks are not included in the licence.

249. FMT or other faecal-derived preparations have been used as an experimental treatment against IBD. Changes in the host-microbiome profile has been determined to last at least 300 days in healthy volunteers that have received FMT (Goloshchapov et al., 2019). Donors of FMT are usually healthy volunteers and the donor material would be screened for a wide range of bacterial and parasitic infections. Hence, it is unlikely that FMT donor material would contain pathogenic bacterium. The applicant has proposed a 6-month exclusion period for people who have received FMT. Therefore, the likelihood of the GMO producing a novel pathogenic bacterium resulting from FMT is unlikely. However, as a conservative measure, a licence condition has been included in the licence to exclude participants that have received FMT or other faecal derived preparation to further limit the potential introduction of other pathogenic bacteria in the gut.

250. The GMO may be shed in faeces or vomit. Although there is the potential that *E. coli* may survive wastewater treatment as discussed in Chapter 2, Section 3.2, the shedding of the GMO is expected to be limited (small number of patients); diluted in a large volume of wastewater; have a reduced viability in the broader environment, and have limited potential to cause harm, and therefore a requirement to decontaminate the toilets after use is not included in the licence. A licence condition has been included in the licence requiring the licence holder to provide written instructions to trial participants regarding decontamination of faeces/diarrhoea or vomit, and to obtain written agreement that trial participants will comply with these instructions to limit the dispersal of the GMO.

251. As discussed in Chapter 1, Section 5.2, infants often acquire part of their microbiome from their mothers, including through breast milk and babies born vaginally have a gut microbiome similar to the mother's birth canal. Therefore, there is some possibility of exposure of infants to the GMO during breastfeeding and when giving birth. Additionally, the gut of an infant is often more easily colonised than adults and antibiotic use is higher in young children (Yang et al., 2016). Therefore, this risk would be minimised by excluding breastfeeding and pregnant women and a conservative approach has been taken given some areas of uncertainty about the GMO, and as such a condition to exclude pregnant and breastfeeding women from the clinical trial has been included in the licence.

252. The clinical staff preparing and observing the administration of the GMO of participants would wear PPE including gown and gloves. Additional PPE (masks and eye protection) would be worn when cleaning any accidental spills or potential shedding of the GMO via contaminated faeces, vomit or rupture of samples with decontaminants that are effective against the GMO. These practices would minimise exposure of people handling and administering the GMOs (Risk scenario 1) and have been included in the licence conditions.

253. Conditions are included in the licence requiring the licence holder to ensure that all GMOs within the clinical trial site, including material or waste that has been in contact with the GMO, are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Licence conditions require the licence holder to ensure that the GMO, or material or waste that has been in contact with the GMO, to be destroyed by external service providers is done through a clinical waste stream. This is considered satisfactory, provided that the licence holder is only permitted to engage persons who can adhere to appropriate standards to conduct the dealings.

254. The Industry Code of Practice for the Management of Clinical and Related Wastes details requirements for clinical waste including waste segregation, packaging, labelling, storage, transport and accountability (Biohazard Waste Industry, 2010). The clinical waste stream typically involves destruction of infectious waste by incineration or autoclaving, which are considered appropriate for

disposal of the GMO. Given that *E. coli* can persist in the environment, disposal measures such as burial or maceration would not ensure containment. Therefore, the licence requires waste disposal by external service providers to be by autoclaving or high-temperature incineration. These measures would limit the exposure of people or other animals to the GMOs.

255. A standard condition is included in the licence requiring that the licence holder to ensures dealings are conducted so as to ensure containment of the GMO, not compromise the health and safety of people and minimise unintentional exposure to the GMO. A note to the condition explains that compliance may be achieved by only engaging persons who are required to adhere to appropriate standards to conduct the dealings.

256. Other 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, other than external service providers, of applicable licence conditions.

257. Further conditions to be implemented in the licence is to ensure that a compliance management plan is in place for each clinical trial site before administration of the GMOs commences at that site. The compliance management plan must detail how the licence holder intends to comply with the licence conditions, including listing persons responsible for site management, proposed reporting structures, staff training procedures and transport and disposal processes.

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

258. Licence conditions have been imposed to limit and control the proposed clinical trial, based on the above considerations. These include requirements to:

- limit the trial to 36 trial participants;
- conduct the trial at suitable clinical trial sites;
- limit the time when the GMO can be administered to 5 years from issue of the licence;
- restrict access to the GMO;
- ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements;
- ensure appropriate PPE is used;
- restrict personnel permitted to administer the GMO;
- appropriately decontaminate the GMO and materials and equipment that have been in contact with the GMO;
- transport and store the GMO and samples from GMO-treated participants in accordance with IATA shipping classification UN 3245 or UN 3373 [Category B] and/or the minimum requirements for packaging, and labelling as detailed in the licence;
- use the clinical waste stream when external service providers are used to destroy unused GMO and GMO-related waste.

3.2 Other risk management considerations

259. All DIR licences issued by the Regulator contain several 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

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

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

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

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

3.2.2 Contingency plans

263. Melius is required to submit a contingency plan to the Regulator before commencing dealings with the GMOs. This plan would detail measures to be undertaken in the event of:

- the unintended release of the GMOs, including spills
- exposure of, or transmission to persons other than trial participants
- a person exposed to the GMOs developing a serious adverse response.

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

264. The persons covered by the licence would be 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 dealings with the GMOs, Melius is required to provide a list of people and organisations that are covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

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

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

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

- identification of the clinical trial sites where administration of the GMO to trial participants would take place
- expected date of administration with the GMOs for each clinical trial site
- cease of administration with the GMOs for each clinical trial site.

3.2.5 Monitoring for compliance

267. 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.

268. 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.

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

Section 4 Issues to be addressed for future releases

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

- information and data that would address the uncertainties noted in Chapter 2, Section 4. Specifically, information obtained on the biodistribution and shedding of the GMOs in trial participants and the potential of the GMO to cause genotoxicity.

Section 5 Conclusions of the RARMP

271. The risk assessment concludes that the proposed clinical trial of the GMOs poses negligible to low risks to the health and safety of people or the environment as a result of gene technology.

272. The risk management plan concludes that the identified negligible to low risks can be managed to protect the health and safety of people and the environment. Conditions are imposed to limit the trial to the proposed scale, location and duration, and to restrict the spread and persistence of the GMOs and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks. Specific risk treatment measures are proposed in the licence to manage the risk of exposure to the GMO and novel bacteria.

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

The Regulator received a number of submissions from prescribed experts, agencies and authorities[2] on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Submission	Summary of issues raised	Comment
1	Does not agree:	
	• That the risk assessment identifies all plausible risk scenarios by which the proposed dealings could potentially give rise to risks relating to the health and safety of people or the environment, specifically related to Risk scenario 2.	The risk scenarios have been reassessed and Risks scenarios 2 and 3 have been further characterised as substantive risks in Chapter 2, Section 3. Risk scenario 2 is related to horizontal gene transfer (HGT) from the GMO to other gut bacteria.
	• That the limits and controls proposed in the draft licence to prevent the spread of the GMO are appropriate for the trial, specifically related to Risk scenario 3.	Risk scenario 3 determined that the key route of exposure is via faecal oral transfer through poor hand hygiene, during sexual activity with participants receiving the GMO, or through inadequate cleanup of vomit and diarrhoea. The overall risk estimates for these risk scenarios are considered to be negligible to low. Conditions to manage the substantive risks are discussed in Chapter 3, Section 2 of the RARMP.
	• With the overall conclusion of the RARMP	Following further risk characterisation of 2 of the risk scenarios, the Regulator has concluded that the overall estimated risk to be negligible to low. Risk mitigation measures are included in the licence conditions to manage these risks.

[2] Prescribed experts, agencies and authorities include the Gene Technology Technical Advisory Committee, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	The Regulator should:	
	• Consider clarifying licence condition wording aiming to reduce faecal-oral contact/transmission.	The licence condition has been amended to ensure that the intent to prevent faecal-oral transmission from sexual activities is clear. A licence condition requiring the licence holder to obtain written consent from participants to comply with good hand hygiene practices is already included in the licence.
	• Consider whether the uncertainty in the pre-clinical data is sufficient to support that the likelihood of transmission of *ttr* operon to pathogenic bacteria is negligible or low.	Risk scenario 2 (Chapter 2, Section 3.1 of the RARMP) discusses the likelihood of horizontal gene transfer of *ttr* operon to other pathogenic bacteria. Further consideration of Risk scenario 2 has determined that the overall risk estimate for this risk scenario is negligible to low.
	• Further consider clarifying the exclusion criterion relating to taking probiotics and whether probiotics should be defined.	Probiotics are not known to be pathogenic and any transfer of genetic material from the GMO is unlikely to result in harm, as considered in Chapter 3, section 3.1.1. Therefore, the condition requiring exclusion of people that have used probiotics has been removed from the licence.
2	No advice or comments on the risk management plan for DIR-221.	Noted.
3	Noted the description of the GMO and the details of the clinical trial. Agrees that the risks to health and safety of people or the environment from the proposed dealings are negligible. Advised that:	Noted.
	• Training of participants in hand hygiene needs to be very thorough especially for food preparation; and	A licence condition is included to ensure that the licence holder obtain written agreement from the trial participant that they will agree to comply with good hand hygiene practices.
	• Participants should be monitored until they have evidence that they are no longer shedding the GMO, which could take longer than 28 days.	Samples will be collected as part of the clinical trial. The RARMP concluded that the risk from the shedding of the GMO or novel bacteria to be negligible to low.

Submission	Summary of issues raised	Comment
		Licence conditions have been included to obtain agreement from trial participants to undertake several hygiene measures to minimise transmission of the GMO. No additional conditions were imposed on the period of sample collection.
4	Sought clarification whether additional controls will be in place for patients who may commence additional treatments during the trial, such as antibiotics and laxatives that could potentially increase the risk of shedding of the GMO. Recommends that the RARMP be amended to ensure the applicant: • take appropriate steps to ensure trial participants are informed of the need to report use of such additional treatments; and	Licence conditions are included to exclude trial participants that use agents that can alter gut transit time and those who are currently using antibiotics. The RARMP has been amended to clarify that if required by a participant during the trial period, antibiotic use is likely to eliminate the GMO in participants, and no increased shedding of the GMO is expected. Licence conditions have been included to obtain agreement from trial participants to undertake several hygiene measures to minimise transmission of the GMO. These measures are expected to be effective, even in the event of increased GMO shedding during the trial.
	• implements measure to effectively manage and monitor these participants to safeguard the integrity of the trial results.	The OGTR assesses the risk of exposure of the GMO to people and the environment. The integrity of the trial results is outside the remit of the OGTR.
5	Had the following comments: • satisfied that the discussion in the RARMP was extensive; • acknowledged that the GMO can be shed into the environment; and • agree that the GMO is not pathogenic but could potentially become pathogenic from HGT. However, the risk is reduced due to the modification on the GMO.	Noted.