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

Risk Assessment and Risk Management Plan for

DIR 197

Clinical trial of genetically modified *Lactobacillus brevis* for treatment of inflammatory bowel disease

Applicant: Novotech (Australia) Pty Ltd

21 September 2023

Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 197

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for a clinical trial using a genetically modified organism (GMO). It qualifies as Dealings involving the Intentional Release (DIR) of genetically modified organisms into the Australian environment under the *Gene Technology Act 2000* (the Act).

The applicant, Novotech (Australia) Pty Limited (Novotech) proposes to conduct a first-in-human clinical trial of genetically modified (GM) *Lactobacillus brevis* bacteria for treatment of inflammatory bowel disease. The GMO would be administered orally and is designed to have anti-inflammatory effects in the gastrointestinal tract.

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, Novotech will require authorisation from the 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. Novotech will also require approval from the Department of Agriculture, Fisheries and Forestry for import of the GM treatment.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which was finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that the proposed clinical trial poses negligible to moderate risks to human health and safety and the environment, and that the risks posed by the dealings can be managed by imposing conditions on the release.

The licence

Project title	Clinical trial of genetically modified *Lactobacillus brevis* for treatment of inflammatory bowel disease
Parent organism	*Lactobacillus brevis*
Genetic modifications[1]	• Introduction of gene encoding human vasoactive intestinal peptide (VIP) to reduce inflammation
Principal purpose	To assess the safety of single and multiple ascending doses of the GMO in healthy clinical trial participants
Previous clinical trials	None
Limits and controls	
Duration	7 years

[1] Information about genetic modifications other than the introduction of the VIP gene is protected as Confidential Commercial Information (CCI). Under Section 185 of the Act, the confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Release size	Up to 28 trial participants will be treated with the GMO
Locations	Medical facilities and the homes of clinical trial participants in Melbourne, Victoria
Controls	• importing the GMO in a form that is double packaged and ready for administration • tracking GMO doses dispensed to clinical trial participants and destroying any GMO doses that remain unused at the end of the trial • issuing spill kits to trial participants to clean up any spill of GMO that occurs at home • instructing clinical trial participants in appropriate hygiene measures

Risk assessment

The risk assessment concludes that the proposed clinical trial poses negligible to moderate risks to human health and safety and the environment. Specific risk treatment measures are included in the licence to manage these risks.

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

Credible pathways to potential harm that were considered include potential exposure to the GMO through accidental ingestion or through shedding from trial participants; the potential for the introduced gene to be transferred to other bacteria; and the potential for the GMO to spread in the environment and enter food and feed.

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

- the GMO is not expected to colonise human or animal guts;
- the small scale of the clinical trial minimises the likelihood of horizontal gene transfer events;
- there are plausible pathways for release of the GMO into the outdoor environment;
- there is uncertainty regarding the ability of the GMO to establish and spread in the environment;
- VIP is capable of causing adverse health effects at sufficiently high levels of exposure.

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 moderate risks can be managed to protect the health and safety of people and the environment by imposing specific risk treatment measures. A number of licence conditions are imposed to restrict release of the GMO into the outdoor 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

CCI	Confidential Commercial Information
CFU	Colony forming unit
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
GM	Genetically modified
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal gene transfer
HREC	Human Research Ethics Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
kg	kilogram
mg	milligram
mL	millilitre
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
NSQHS	National Safety and Quality Health Service
OGTR	Office of the Gene Technology Regulator
pmol/L	Picomoles per litre
QPS	Qualified Presumption of Safety
RAF	Risk Assessment Framework
RARMP	Risk Assessment and Risk Management Plan
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
VIP	Vasoactive intestinal peptide

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

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

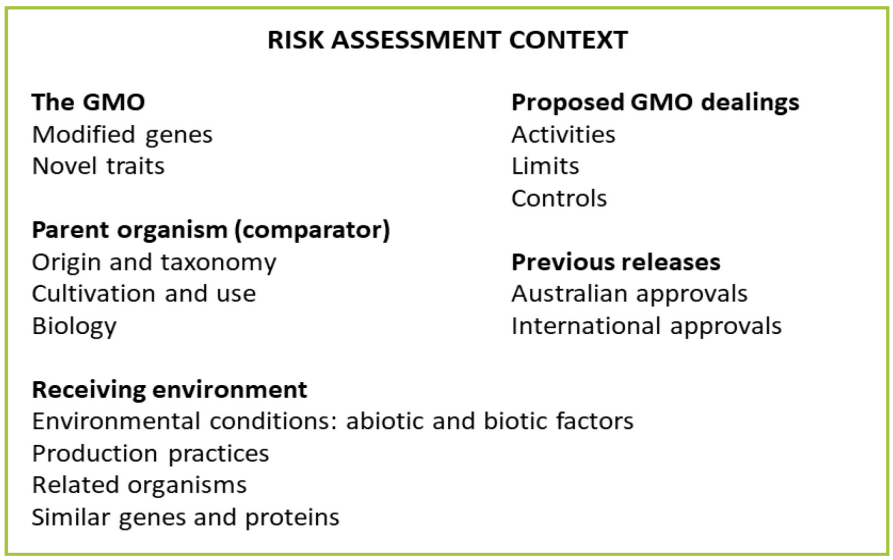

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

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

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, the Australian Pesticides and Veterinary Medicines Authority, the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Fisheries and Forestry (DAFF).

9. The DAFF regulates products imported into Australia to protect Australia from biosecurity risks. Under the *Biosecurity Act 2015*, the importation of biological material such as live GM treatments requires a permit from the DAFF.

10. 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 scheme or the Clinical Trial Notification scheme.

11. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HREC review is a part of the research governance process carried out by an institution that is responsible for the quality, safety and ethical acceptability of research carried out under their auspices. HRECs review research proposals involving human participants to ensure that they are ethically acceptable and meet relevant standards and guidelines. Elements of research to be considered include research merit and integrity, justice, beneficence, and participant consent.

12. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on Ethical Conduct in Human Research, 2018* (National Statement) (National Health and Medical Research Council et al., 2018) which is the principal ethics guideline setting out the requirements for the ethical design, review and conduct of human research in Australia. The *Therapeutic Goods Act 1989* requires an HREC to review and monitor all clinical trials of unregistered therapeutic goods. The HREC must be registered with the NHMRC and constituted and operating in accordance with the National Statement.

13. In terms of risk to individuals participating in a clinical trial, the TGA (as the primary regulatory agency of investigational products), the trial sponsor, the investigators and the HREC responsible for each trial site all have roles in ensuring participant's safety under the *Therapeutic Goods Act 1989* and the requirements of the National Statement. 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 GMO, and risks associated with import, transport and disposal of the GMO.

14. The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guideline for Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the

participation of human subjects (ICH, 2016). The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States of America, as well as those of Australia, Canada, the Nordic countries and the World Health Organization. The TGA has adopted the Integrated addendum to ICH E6(R1): Guideline for good clinical practice E6(R2) (Therapeutic Goods Administration), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

15. Some dealings with the GMO will be conducted at clinical trial sites, which are medical facilities including out-patient settings, hospitals and associated pharmacies. Analysis of biological samples collected from trial participants administered with the GMO may occur at clinical trial sites or at pathology laboratories.

16. 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 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.

17. The National Pathology Accreditation Advisory Council (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 category, by an Approved Pathology Practitioner employed by an Approved Pathology Authority. 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.

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, 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. Novotech (Australia) Pty Ltd (Novotech) is seeking authorisation to carry out a clinical trial of a genetically modified (GM) *Lactobacillus brevis* (LIV001) for treatment of inflammatory bowel disease. The purpose of the proposed first-in-human study is:

(a) to assess the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of the GMO in healthy clinical trial participants, and

(b) to assess the safety, tolerability, and efficacy of multiple doses of the GMO in clinical trial participants with mild-to-moderate active ulcerative colitis.

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

(a) import the GMO;

(b) conduct the following experiments with the GMO:

i. oral administration of the GMO to clinical trial participants;

ii. collection of samples from trial participants;

iii. analysis of samples from trial participants;

(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 Proposed limits of the trial

21. The clinical trial is proposed to take place over a seven-year period from the date of issue of the licence.

22. Up to 60 people are proposed to be enrolled in the clinical trial. The treatment duration would range from a single dose of the GMO to daily doses of the GMO over a period of 8 weeks.

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

23. The applicant has proposed a number of controls to restrict the spread and persistence of the GMO in the environment. These include:

- importing the GMO in a form that is double packaged and ready for administration[2];
- tracking GMO doses that have been dispensed to clinical trial participants for self-administration at home and destroying any GMO doses that remain unused at the end of the trial;
- issuing spill kits to trial participants to clean up any spill of GMO that occurs at home;
- instructing clinical trial participants in appropriate hygiene measures, such as hand washing after using the toilet;
- only enrolling trial participants who agree to abstain from unprotected anal sex.

2.3 Details of the proposed dealings

2.3.1 Manufacture and import of the GMO

24. The GMO will be produced by an Australian pharmaceutical company under a separate authorisation and exported to the United Kingdom for manufacture of the drug product. The GMO will be imported in a form that is ready for administration in the clinical trial. Import of the GMO will be conducted in accordance with International Air Transport Association (IATA) guideline UN3245 (GMOs that are not classified as category A or B infectious substances).

[2] Some information about the dosage form and packaging of the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

2.3.2 Trial design

25. The proposed clinical trial will be conducted in three sequential parts. In all three parts of the trial, participants would be randomised to receive GMO or placebo at a ratio of 2:1.

26. Part A of the proposed clinical trial is a single ascending dose study that will enrol 18 healthy adults to receive a single dose of either the GMO or placebo. Half of the participants receiving the GMO would receive a low dose and half of the participants would receive a high dose (10 x low dose)[3].

27. Part B of the proposed clinical trial is a multiple ascending dose study that will enrol 18 healthy adults to receive a daily dose of either the GMO or placebo for 14 days. Half the participants receiving the GMO would receive a low dose and half of the participants would receive a high dose (10 x low dose).

28. Part C of the proposed clinical trial is a multiple dose study in adult patients with ulcerative colitis, a form of inflammatory bowel disease. Approximately 15 patients with mild-to-moderate ulcerative colitis would receive a medium dose (5 x low dose) of either the GMO or placebo daily for 56 days.

29. The proposed trial design is summarised in Table 1.

2.3.3 Administration of the GMO

30. The GMO doses would be self-administered orally by trial participants.

31. In Parts A and B of the proposed clinical trial, the trial participants would remain in a clinical trial facility for the first three days of the trial. For Parts A and B of the trial, the clinical trial facility would be Nucleus Network Melbourne Clinic.

32. In Part A of the trial, the trial participants would take their only dose of the GMO at a clinical trial facility under the supervision of staff.

33. In Part B of the trial, the trial participants would take their first three daily doses of the GMO at a clinical trial facility under the supervision of staff. The remaining eleven daily doses would be dispensed to the trial participants to self-administer at home.

34. In Part C of the trial, the trial participants would take their first daily dose of the GMO at a clinical trial facility under the supervision of staff. The remaining 55 daily doses would be dispensed to the trial participants to self-administer at home. For Part C of the trial, multiple clinical trial facilities may be used.

[3] Some information about the dosage form of the GMO and the quantity of GMO in each dose is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

Table 1 Summary of clinical trial design

	People receiving GMO	People receiving placebo	Dose level	Number of doses	Doses at clinical trial facility	Doses at home
Part A	12	6	1 (50%) 10 (50%)	1	1	0
Part B	12	6	1 (50%) 10 (50%)	14	3	11
Part C	~10	~5	5	56	1	55

2.3.4 Selection of trial participants

35. Relevant inclusion criteria proposed by the applicant include:

- participants must be willing and able to comply with all study-related procedures and assessments; and
- for Parts A and B of the study, participants must be generally healthy, based on medical history and tests conducted at screening; and
- for Part C of the study, participants must have active mild-to-moderate ulcerative colitis at Day 1 of the trial.

36. Relevant exclusion criteria proposed by the applicant include:

- women who are pregnant or lactating; and
- for Parts A and B of the study, functional gastrointestinal disorders, e.g., irritable bowel syndrome, heartburn, nausea or dyspepsia; and
- for Part C of the study, history of a condition associated with significant immunosuppression.

2.3.5 Sample collection and analysis

37. Blood, urine and stool samples will be collected from trial participants for analysis. Blood and urine samples will be collected during visits to a clinical trial facility. Stool samples will be collected either at a clinical trial facility (during the period that participants remain in the clinical trial facility) or at home. Clinical trial staff would provide the trial participants with commercial collection kits for stool samples. Some stool samples (for safety examinations) would be collected without processing, and could contain viable GMO. Other stool samples (for pharmacokinetics assessment) would be collected in sample tubes that homogenise and preserve samples, and are not expected to contain viable GMO. Stool samples that contain GMO are proposed to be analysed on-site at the clinical trial facilities.

2.3.6 Transport and storage of the GMO

38. GMO doses stored at clinical trial sites would be handled in accordance with the Regulator's *Guidelines for Transport, Storage and Disposal of GMOs* for risk group 1 organisms.

39. GMO doses dispensed to trial participants would be transported by the trial participants from the clinical trial sites to their homes by their usual mode of transport. The GMO doses would be

dispensed double-packaged[4]. GMO doses would be dispensed to trial participants during site visits: twice for participants in Part B of the trial and three times for participants in Part C of the trial. Trial participants may also transport GMO doses if they travel during the period of the clinical trial or if they return unused doses to the clinical trial sites.

40. GMO doses dispensed to trial participants would be stored at the trial participants' homes. If the participants travel and stay away from home during the clinical trial, the GMO doses would also be stored at temporary accommodation[5]. Trial participants would be instructed to store GMO doses in a fridge.

41. Stool samples containing GMO would be transported by the trial participants from their homes to clinical trial sites.

2.3.7 Disposal of the GMO

42. At the clinical trial sites, unused GMO or waste containing GMO would be disposed of via the clinical waste stream.

43. Trial participant stool containing GMO would be released into the normal sewage system.

2.3.8 Accountability and Monitoring

44. A record of the quantity of GMO dispensed to each trial participant would be maintained. Each trial participant would self-administer doses of the GMO at home and record the details in a diary. Compliance with the prescribed dosage regime would be assessed at each clinical trial site visit by reviewing the diary. On completion of the study, any unused GMO doses would be returned to the clinical trial site for disposal. Evidence of the use of all dispensed GMO or the destruction of any surplus GMO would be documented.

2.3.9 Contingency plans

45. In the event of a spill of GMO at a trial participant's home, the trial participant would be instructed to use a spill kit to clean up the GMO, place the collected GMO and materials used to clean the spill in a sealable bag and return the sealable bag to a clinical trial site for appropriate disposal[6].

46. Trial participants would keep a diary to track use of the GMO at home. Any accidental ingestion of the GMO by a person other than a trial participant would be reported to Novotech and the OGTR.

47. If treatment of the GMO became necessary, the applicant states that effective antibiotics could be administered[7].

Section 3 Parent organism

48. The parent organism of the GMO is *Lactobacillus brevis*, also known as *Levilactobacillus brevis*, which belongs to the *Bacillus* class of the *Firmicutes* phylum of bacteria (Zheng et al., 2020). The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with the GMO. The relevant biological properties of *L. brevis* will be discussed here.

[4] Some information about the packaging of the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

[5] Some information about the timing of GMO administration is protected as CCI.

[6] Some information about the GMO dosage form is protected as CCI.

[7] Information about specific antibiotics that are effective against the GMO is protected as CCI.

49. *L. brevis* is a gram-positive, facultatively anaerobic, rod-shaped bacteria that does not produce spores. It is a heterofermentative lactobacteria, meaning that its main source of energy is fermentation of sugars into lactic acid, CO_2 and either acetic acid or ethanol (Schleifer, 2009; Zheng et al., 2020).

3.1 Risk group

50. The Australian Standard for microbiological safety and containment defines four risk groups for microorganisms (Standards Australia and Standards New Zealand, 2022). *L. brevis* is not a listed organism in the Standard, which only lists microorganisms from Risk Group 2 or higher. According to the Public Health Agency of Canada ePATHogen - Risk Group Database, *L. brevis* is Risk Group 1. Similarly, according to the German government Central Committee on Biological Safety Database of safety-assessed microorganisms, *L. brevis* is Risk Group 1. Risk Group 1 classification is given, internationally, to microorganisms that are unlikely to cause human or terrestrial animal disease (Standards Australia and Standards New Zealand, 2022).

51. The European Food Safety Authority (EFSA) maintains a list of microorganisms which have received Qualified Presumption of Safety (QPS) status for intentional addition to food and feed. *L. brevis* has QPS status, with the qualification that strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antimicrobials (EFSA Panel on Biological Hazards et al., 2023). *L. brevis* strains are used commercially as starter culture for fermentation of human food and animal feed (Zheng et al., 2020). *L. brevis* is also present as a minor component in many commercial probiotic products (Morovic et al., 2016). The Australian Register of Therapeutic Goods currently lists 13 probiotic products containing *L. brevis*, including products intended for children (TGA website, accessed 7/7/2023).

52. The Risk Group 1 classification and QPS status of *L. brevis* indicate that it is not considered pathogenic or harmful to humans or animals.

3.2 Habitat

53. Members of the *Lactobacillus* genus are found in nutrient-rich habitats. A study of their lifestyles assigned *Lactobacillus* species into three categories: free-living (associated with plant material or environment), host-adapted (associated with invertebrate or vertebrate hosts) or nomadic. *L. brevis* was assigned to the free-living lifestyle group (Duar et al., 2017). This means that *L. brevis* is an environmental bacterium that does not normally colonise human or animal guts.

54. *L. brevis* is found at low levels on plant surfaces and grows abundantly in decaying plant material (Schleifer, 2009). It occurs widely in vegetable and cereal fermentations (Zheng et al., 2020). For instance, it is a component of the fermentation cultures for silage, sourdough, sauerkraut and other pickled vegetables, and it is a problematic spoilage organism for beer (Schleifer, 2009; Feyereisen et al., 2019; Ashaolu and Reale, 2020; Zheng et al., 2020). *L. brevis* also grows well on domestic kitchen and garden waste (Probst et al., 2013).

55. Globally, *L. brevis* is ubiquitous in the environment (Rychen et al., 2016). A study of *Lactobacillus* distribution on food plants in subtropical North America found *L. brevis* on 6 of the 12 plant types sampled (Mundt and Hammer, 1968). A study of lactic acid bacteria on forage plants in temperate South America found *L. brevis* on 6 of the 14 plant species sampled (Puntillo et al., 2020). Even a study of lactic acid bacteria on forage plants in the cold and arid Tibetan plateau found *L. brevis* on 1 of the 5 plant species sampled (Pang et al., 2012). The measured abundance of *Lactobacillus* species on fresh food plant samples was up to 500 CFU/g (Mundt and Hammer, 1968), and on fresh forage plant samples was up to 2000 CFU/g (Pang et al., 2012), which were both very small proportions of total bacterial abundance. However, lactic acid bacteria that are present in very low numbers in raw plants can reach 10^6 to 10^8 CFU/g within a few days of incubation in plant-based food and beverage fermentations (Yu et al., 2020).

56. *L. brevis* is reported to be occasionally recovered from intestines of humans, pigs, birds, cattle and rats (Schleifer, 2009). A large metagenomic analysis of *Lactobacillus* species prevalence in human faecal samples found that *L. brevis* genomes were present with a relative abundance of greater than 0.01% in 0.4% of faecal samples from healthy individuals (Ghosh et al., 2020). A similar study by a different group of researchers detected *L. brevis* genomes in 0.6% of human stool samples, with a median relative abundance of 0.016% (Pasolli et al., 2020).

57. Most lactobacteria present in the human gastrointestinal tract are regarded as transient members of the gut microbiota, that are temporarily present following ingestion of fermented food (Pasolli et al., 2020). A large study of the effect of fermented food consumption on the human gut microbiome found that the relative faecal abundance of a set of *Lactobacillus* bacteria associated with fermented food (*L. brevis* and seven other species) was over 50-fold higher in people who regularly consumed fermented plants than in people who rarely or never consumed fermented plants (Taylor et al., 2020). The low prevalence and abundance of *L. brevis* in human faecal samples, combined with the clear link between *L. brevis* presence and consumption of fermented food containing lactobacteria, strongly suggest that ingested *L. brevis* does not colonise and persist in the human gastrointestinal tract.

58. A study of *L. brevis* as a probiotic found that the two tested strains of *L. brevis* can survive and multiply under a regime of 3 hours in simulated human gastric juice and 7 hours in simulated human intestinal juice (Fukao et al., 2013). The survival rates of the two strains were approximately 110% and 220% of the *L. brevis* cells ingested. This study did not simulate competition with gut microorganisms, which might reduce survival rates to some extent. However, a high proportion of *L. brevis* ingested is expected to survive transit through the gastrointestinal tract.

3.3 Infections and control

59. Infections caused by *Lactobacillus* species are very rare, but occasionally occur in people with underlying medical conditions (Schleifer, 2009; Rossi et al., 2019). For instance, in a large study of bacteremia cases in Finland, 0.2% of cases were caused by *Lactobacillus* species, and most of the patients infected with *Lactobacillus* species had a severe underlying condition (organ transplant with immunosuppressive treatment or metastatic cancer) (Saxelin et al., 1996). The most common types of infections caused by *Lactobacillus* species are bacteremia and endocarditis (Cannon et al., 2005; Rossi et al., 2019). In a review of *Lactobacillus*-associated infections, one of 140 cases where the species was identified was caused by *L. brevis* (Cannon et al., 2005).

60. Three recent studies of antibiotic resistance in *Lactobacillus* species tested a total of 17 strains of *L. brevis*. All strains of *L. brevis* were susceptible to the antibiotics chloramphenicol and erythromycin and almost all strains were susceptible to ampicillin and clindamycin (Anisimova and Yarullina, 2019; Dušková et al., 2020; Stefanska et al., 2021).

61. *Lactobacillus* species are resistant to inactivation by acid at pH 2 and by alkali at pH 12. They are inactivated by heat treatment at 80°C for 15 minutes or 100°C for 5 minutes (Almada et al., 2021). *L. brevis* strains are susceptible to the biocides benzalkonium chloride, triclosan and chlorhexidine. They are moderately susceptible to the biocide sodium hypochlorite, with some strains requiring sodium hypochlorite concentrations of 2 – 4 mg/mL for inactivation (Arioli et al., 2013).

3.4 Horizontal gene transfer

62. A comparative genome analysis of 19 *L. brevis* strains found that they contained an average of 5 plasmids per strain (Feyereisen et al., 2019). A study of a *L. brevis* strain containing 9 plasmids found that one plasmid contained a full set of the genes required for conjugation (Fukao et al., 2013). Other plasmids are likely mobilizable and capable of horizontal transfer between bacteria during a conjugation process.

63. The genomes of *L. brevis* strains were found to contain from 1 – 7 prophage integration sites, with an average of 3 prophage integrations per strain, about half of which were intact prophages (Feyereisen et al., 2019). This indicates that *L. brevis* is susceptible to infection by prophages, and these prophages may be able to horizontally transfer DNA between bacterial genomes.

64. The chromosomes of *L. brevis* strains contain between 2088 and 2674 protein coding sequences (Feyereisen et al., 2019). Some of the chromosomal genes associated with survival of strains in particular environmental niches are reported to be acquired by horizontal gene transfer (Romano et al., 2014; Feyereisen et al., 2019).

3.5 Parental strain

65. The name and some information about the parental strain is protected as confidential commercial information (CCI). Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

66. It is unknown whether the parental strain is present in Australia.

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

4.1 The genetic modifications and effects

67. The GMO was developed by Liveome Inc and is called LIV001. LIV001 is GM *L. brevis* with an introduced gene cassette encoding vasoactive intestinal peptide (VIP). One rationale for introducing VIP into *L. brevis* is to combine two treatments (i.e. the probiotic effect of *L. brevis* and the immunomodulatory effect of VIP) that may have a positive effect in people suffering from inflammatory bowel disease. The other rationale is to provide an extended release formulation for VIP in the gastrointestinal tract.

68. Information about genetic modifications other than the introduction of the VIP gene is protected as CCI. Information about the method of genetic modification is also protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

69. Presence of the intended genetic modifications and absence of any unintended insertions of exogenous sequence were confirmed by whole genome sequencing.

4.1.1 Introduced VIP gene

70. The introduced gene cassette contains a synthetic gene sequence encoding a peptide based on human vasoactive intestinal peptide (VIP). The amino acid sequence of the peptide was modified to enhance stability. In addition, the DNA sequence encoding the peptide was codon-optimised for expression in *Lactobacillus* bacteria.

71. Human VIP is a 28-residue neuropeptide secreted by neurons and immune cells. It regulates multiple physiological functions in organs including the heart, lung, thyroid gland, kidney, urinary tract and gastrointestinal tract (Delgado and Ganea, 2013; Iwasaki et al., 2019; Martinez et al., 2019).

72. In the gastrointestinal tract, VIP receptors are found on mucosal cells of the stomach, small intestine and colon, on a range of immune system cells, and on smooth muscle cells (Iwasaki et al., 2019). Therefore, if the GMO is ingested, the VIP produced can act directly on VIP receptors in the gastrointestinal tract. The VIP would not need to be absorbed into the bloodstream to have a biological effect.

73. VIP homologues are present in a range of vertebrates. VIP is known to have immunomodulatory effects in mammals (Smalley et al., 2009). The intended function of VIP in the GMO is to reduce inflammation in patients with inflammatory bowel disease. Mammalian VIP inhibits

the production of pro-inflammatory cytokines and chemokines and stimulates production of anti-inflammatory cytokines (Delgado and Ganea, 2013; Martinez et al., 2019).

74. As suppression of inflammation in the gastrointestinal tract is the intended therapeutic effect of VIP in the proposed trial, it is not considered an adverse effect for trial participants. However, immunosuppression by VIP would be an adverse effect on health for people other than trial participants.

75. Other known functions of VIP in the gastrointestinal tract include:

(a) vasodilation of the gastrointestinal mucosa;

(b) promoting gastrointestinal motility and reducing food transit time;

(c) stimulating water and anion secretion into the intestines; and

(d) inhibition of gastric acid secretion (Iwasaki et al., 2019).

76. Hyperexpression of VIP, which occasionally occurs in humans due to VIP-secreting tumours, causes high-volume watery diarrhea (Ghaferi et al., 2008; Iwasaki et al., 2019). The associated dehydration and loss of electrolytes may require hospitalisation. Chronic potassium deficiency caused by this condition can be life-threatening if untreated. A hospitalised patient can be stabilised by intravenous rehydration and electrolyte replacement, but the diarrhea will continue as long as high levels of VIP are present (Ghaferi et al., 2008).

77. Intravenous infusion of VIP in healthy adults is reported to cause secretory diarrhea similar to the condition caused by VIP-secreting tumours (Kane et al., 1983). The plasma VIP concentration reported to induce secretory diarrhea is 129 ± 40 pmol/L, compared to a normal plasma VIP concentration of about 15 pmol/L.

78. In a phase 3 clinical trial of VIP as an immunomodulatory treatment for COVID-19 patients, three doses of VIP were administered by intravenous infusion, with a final dose of 150 pmol per kg bodyweight per hour for 12 hours. Mild to moderate diarrhea occurred in 40% of patients who received VIP treatment, compared to 11% of the placebo group (Brown et al., 2023). A similar phase 2b/3 clinical trial of intravenous VIP, sponsored by a different organisation, used the same dosage of VIP and reported mild to moderate diarrhea in 33% of the patients who received VIP, compared to 2% of the placebo group (Youssef et al., 2022). A study where VIP was administered at 300 pmol per kg bodyweight per hour for 5 hours to healthy adults found that watery diarrhea occurred in all study participants. The diarrhea was severe in 70% of participants and moderate in 30% of participants (Keller et al., 2005). In summary, infused VIP at levels used in clinical trials sometimes causes diarrhea, and infused VIP at twice the levels used in clinical trials always causes diarrhea, in most cases severe.

79. Cholera patients with profuse watery diarrhea have normal levels of VIP in blood but very high levels of VIP in stool. This suggests that human cholera diarrhea is mediated by increased intestinal production and release of VIP. Patients with cholera may require hospitalisation for severe dehydration and shock (Afroze et al., 2020).

80. Human VIP is stable in solution at low and neutral pH and at different salt concentrations. However, it is very rapidly degraded by proteases in both simulated gastric fluid and simulated intestinal fluid, with a half-life of less than one minute (Cui et al., 2013). The synthetic VIP gene introduced into the GMO encodes a stablilised VIP analogue[8]. The half-life of the synthetic VIP

[8] Some information about the synthetic VIP analogue produced by the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

secreted by the GMO in the human digestive tract is unknown, but based on confidential information, it is likely to be longer than the half-life of human VIP. If so, each molecule of the synthetic VIP analogue is likely to have greater biological effect than a molecule of human VIP, due to the longer time window available for binding receptors.

81. The synthetic VIP sequence secreted by the GMO is unlikely to be allergenic due to its high homology to endogenous human VIP. In addition, the molecular weight of human VIP is 3.3 kDa (Cui et al., 2013), and there is a general consensus that peptides <3.5 kDa do not pose a risk of sensitisation to IgE-mediated allergic reactions (Wang et al., 2022).

82. There are no known previous clinical trials of orally administered VIP.

4.2 Characterisation of the GMO

83. Results of animal studies with the GMO and some bioinformatic analyses of the GMO are protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

4.2.1 Expression of VIP in the GMO

84. Expression levels of VIP in the GMO have not been quantitatively characterised.

4.2.2 Genomic analysis

85. The applicant states that they tested the genome of the GMO for prophages and there were no inducible phages detected in the GMO.

Section 5 The receiving environment

86. The receiving environment forms part of the context for assessing risks associated with dealings with GM micro-organisms (OGTR, 2013). It informs the consideration of potential exposure pathways.

87. The intended primary receiving environment of the GMO is the gastrointestinal tract of clinical trial participants.

88. As the clinical trial will not be conducted in contained facilities, and viable GMO can be shed from trial participants, the GMO could also enter the local environment.

5.1 Presence of related bacterial species in the receiving environment

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

90. Various *Lactobacillus* species are present throughout the digestive system, i.e. inside the mouth, the stomach mucosa and intestines. DNA sequence analysis indicates that on average less than 1% of the bacteria in the distal human gut are *Lactobacilli* (Rossi et al., 2019). A large international analysis of the microbiome of human faecal samples found that 34% of samples from healthy individuals included at least one *Lactobacillus* species that was detected with a relative abundance of greater than 0.01%. Faecal samples from patients with inflammatory bowel disease had a higher *Lactobacillus* prevalence than samples from healthy individuals (Ghosh et al., 2020).

91. *Lactobacillus* species are also widespread in the Australian environment on plant and animal hosts. For example, various Australian studies have reported that *Lactobacillus* species dominate the bacterial community in maize and sorghum silage (Forwood et al., 2019; Hooker et al., 2019), are normal microflora in the broiler chicken gastrointestinal tract (Stephenson et al., 2009), and are common in craft beer (Menz et al., 2010).

5.2 Presence of similar genetic material in the environment

92. As the vasoactive intestinal peptide is highly conserved in mammals (Smalley et al., 2009), VIP gene homologs are widespread in mammalian cells in the environment.

93. The NCBI tblastn algorithm (accessed 12/5/2023) was used to search for translated bacterial DNA sequences homologous to the human VIP amino acid sequence. No significant similarity was found, indicating that no bacteria sequenced in the NCBI database possess VIP genes.

94. The synthetic VIP gene in the GMO, which encodes a stabilised VIP analogue and is codon-optimised for expression in *Lactobacillus* bacteria, is not present in the environment.

Section 6 Previous authorisations

95. The GMO has not been previously authorised for clinical trials or commercial release in any country. The proposed clinical trial would be a first-in-human study.

Chapter 2 Risk assessment

Section 1 Introduction

96. 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 2). 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.

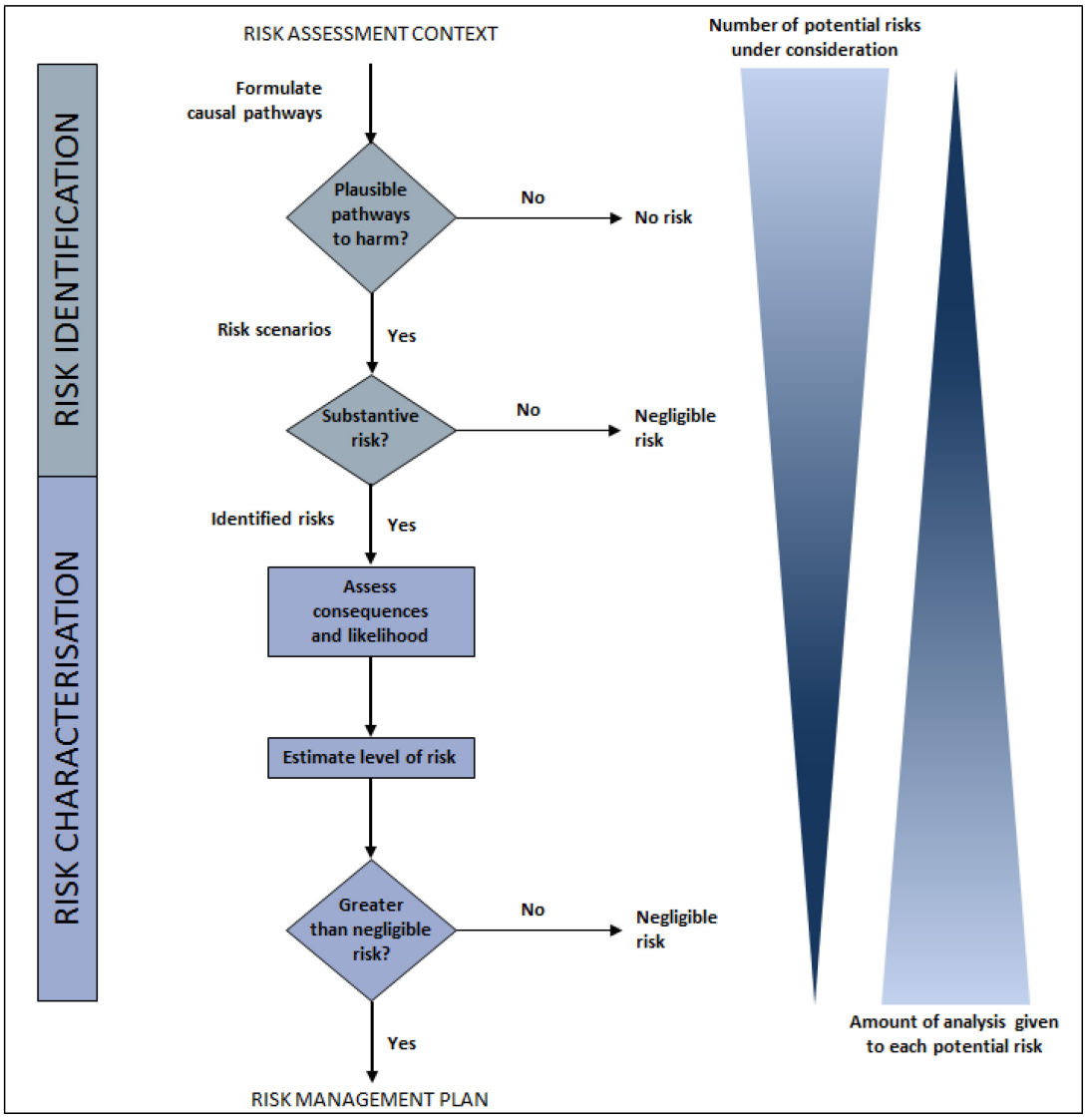

Figure 2: The risk assessment process

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

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

99. 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 2), i.e., the risk is considered no greater than negligible.

100. 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

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

i. the source of potential harm (risk source)

ii. a plausible causal linkage to potential harm (causal pathway)

iii. potential harm to people or the environment.

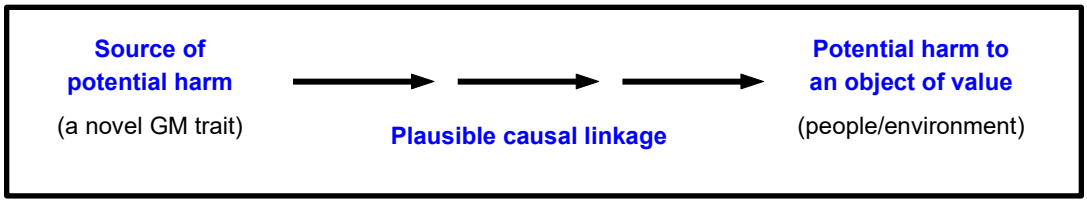

Figure 3: Components of a risk scenario

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

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

103. As discussed in Chapter 1, Section 1, the TGA, the trial sponsor, the Investigators and the 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 on risks posed to people other than clinical trial participants, and to the environment.

2.1 Risk source

104. The sources of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

105. As discussed in Chapter 1, Section 4, the GM *L. brevis* has been modified by inserting a synthetic gene encoding vasoactive intestinal peptide (VIP). This introduced gene is considered further as a potential source of risk.

106. No other genetic modifications will be considered further as a potential source of risk[9].

2.2 Causal pathway

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

- the proposed dealings with the GMO;
- proposed limits, including the extent and scale of the proposed dealings;
- characteristics of the parent organism;
- potential effects of introduced or deleted gene(s) on the properties of the organism;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment;
- the release environment;
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential);
- gene transfer by horizontal gene transfer (HGT); and
- unauthorised activities.

108. The dealing of import of the GMO would be conducted in accordance with the appropriate IATA guideline and will not be considered further.

109. The potential for reversion of the GMO to the parental phenotype is not a plausible pathway to harm because the parent organism is not pathogenic or harmful (Chapter 1, Section 3). Therefore, reversion will not be considered further.

110. 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 by the licence applicant will not be considered further.

2.3 Potential harms

111. The introduced gene encodes VIP, which has biological effects in humans and animals. Therefore, the potential harms that will be considered are:

- harm to the health of people; and
- harm to the health of pets, livestock and Australian wildlife.

2.4 Postulated risk scenarios

112. Five risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 2 and discussed in depth in Sections 2.5 - 2.9.

113. In the context of the activities proposed by the applicant and considering both the short and long term, only Risk Scenario 3 gave rise to a substantive risk which warranted further assessment (characterised in Section 3).

[9] Information about the genetic modifications other than introduction of the VIP gene is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

Table 2 Summary of risk scenarios from the proposed dealings with GM bacteria

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
1	GMO secreting VIP	GMO doses are ingested by people other than trial participants or by pets ⬇ GMO enters gut and secretes VIP	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications	No	• The proposed packaging and controls minimise the potential for people other than trial participants or pets to ingest GMO doses • The GMO is not expected to colonise human or animal guts, so any adverse effect would be transitory
2	GMO secreting VIP	Clinical trial participants shed GMO in stool, vomit and/or saliva ⬇ People other than trial participants are exposed to the GMO ⬇ GMO enters gut and secretes VIP	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications	No	• People would be exposed to GMO at doses too low to cause health effects • The GMO is not expected to colonise the human gut, so any adverse effect would be transitory
3	GMO secreting VIP	GMO is released into the outdoor environment, via loss of GMO doses or shedding of GMO from trial participants ⬇ GMO establishes on plant substrates and spreads in the environment	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR	Yes	• There are plausible pathways for release of the GMO into the outdoor environment • There is uncertainty regarding the ability of the GMO to establish and spread in the environment • VIP is capable of causing adverse health

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
		⬇ People or animals consume the GMO and/or secreted VIP in plant material	Secretory diarrhea and associated health complications		effects at sufficiently high levels of exposure
4	GMO containing VIP gene	GMO is present in gut of clinical trial participants ⬇ VIP gene is horizontally transferred to gut bacteria ⬇ Novel GM gut bacteria secreting VIP persist in clinical trial participants and spread to other people	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications	No	• The small scale of the clinical trial minimises the likelihood of HGT events • GM gut bacteria could be treated with antibiotics
5	GMO containing VIP gene	Clinical trial participants shed GMO in stool, which enters sewage ⬇ VIP gene is horizontally transferred to bacteria in sewage ⬇ Novel GM bacteria survive sewage treatment and are released in treated effluent or biosolids ⬇ Humans or animals are exposed to novel GM bacteria secreting VIP	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications	No	• The small scale of the clinical trial minimises the likelihood of HGT events • As VIP secretion is not expected to increase bacterial fitness, the GM trait would not become fixed in a bacterial population

2.5 Risk scenario 1

Risk source	GMO secreting VIP
Causal pathway	GMO doses are ingested by people other than trial participants or by pets ⬇ GMO enters gut and secretes VIP
Potential harm	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications

Risk source

114. The source of potential harm for this postulated risk scenario is the GMO, which secretes VIP.

Causal Pathway

Ingestion of GMO doses

115. In part A of the clinical trial, all GMO doses would be ingested at a clinical trial site under the supervision of clinical trial staff. However, in parts B and C of the proposed clinical trial, GMO doses would be dispensed to trial participants for self-administration at home. The postulated causal pathway is that in the home environment, GMO doses could be accidentally ingested by other adults, children or pets. The likelihood of this happening is considered below.

116. If a trial participant's home contains another adult who takes medication, the other adult could accidentally take the GMO instead of their intended medication. However, this is highly unlikely, as the GMO would be dispensed in a labelled container that could be easily distinguished from the intended medication.

117. If a trial participant's home contains a young child, and the child has access to the GMO, the child could swallow GMO doses. However, the GMO would be in child-resistant packaging[10]. Trial participants would be instructed to keep the GMO in a fridge, and many fridges are difficult to access for toddlers, although they are easily accessed by older children. In addition, the applicant states that the GMO containers would be labelled "Keep out of reach of children", which could prompt trial participants to take further measures to secure the GMO if needed. Therefore, it is highly unlikely that a child would accidentally ingest GMO doses.

118. If a trial participant's home contains an unconfined pet, such as a dog or cat, and the pet has access to the GMO, the pet could eat GMO doses. However, trial participants would be instructed to keep the GMO in a fridge, and fridges are not accessible by pets. If a trial participant accidentally drops a GMO dose on the floor during self-administration, their pet could eat the GMO, but it is expected that a pet owner would try to stop their pet from eating dropped medication. Therefore, it is highly unlikely that pets could consume GMO doses.

119. Trial participants could spill GMO doses[11]. To manage this risk, the applicant proposes to issue spill kits to trial participants to clean up any spill of GMO that occurs at home. The spilt GMO and

[10] Some information about the packaging of the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

[11] Some information about the dosage form of the GMO is protected as CCI.

materials used for cleaning up the GMO would be placed in a sealed bag and returned to a clinical trial site for disposal. This measure is expected to minimise exposure of people and pets to any spilt GMO.

120. The applicant also proposes to track GMO doses that have been dispensed to clinical trial participants for self-administration at home, and destroy any GMO doses that remain unused at the end of the trial. This measure would prevent trial participants from storing unused GMO at home for long periods after the end of the trial, so it would reduce the likelihood of accidental ingestion of the GMO by people other than the trial participants or pets.

121. Overall, the proposed packaging and controls minimise the potential for people other than trial participants or pets to ingest GMO doses.

Secretion of VIP

122. If people or animals ingest a dose of GMO, the GMO would secrete VIP in their gastrointestinal tracts. However, as discussed in Chapter 1, Section 3, *L. brevis* is an environmental bacterium that is not adapted to live in human or animal hosts, and studies clearly indicate that it does not persist in human gastrointestinal tracts after ingestion.[12] Therefore, the GMO is not expected to colonise human or animal gastrointestinal tracts, and secretion of VIP in the gut would be transitory.

Potential harm

123. The potential harms from the GMO secreting VIP are either suppression of the immune system in the gastrointestinal tract, which would increase susceptibility to pathogen infection and development of disease, or secretory diarrhea and associated health complications. Note that suppression of the immune system in the gastrointestinal tract is the intended therapeutic effect of the GMO, so it is not a harm in trial participants, but it would be a harm in people other than trial participants or animals.

124. As discussed in Chapter 1, Section 4, VIP is a signalling molecule with an anti-inflammatory effect. Inflammation is the initial response of the immune system to pathogens and triggers other steps in the immune response. Therefore, secretion of VIP in the gastrointestinal tract could suppress the local immune system. This could increase susceptibility to infections by pathogens whose portal of entry is the gastrointestinal tract. These infections could lead to a range of diseases.

125. VIP causes broad immunosuppression by a related mechanism to corticosteroids, as both VIP and corticosteroids interfere with the activity of the transcriptional regulators AP-1 and NFκB (Ramamoorthy and Cidlowski, 2016; Martinez et al., 2019). The potential harm of increased infections described above is similar to a well-known side effect of corticosteroid medication prescribed for inflammatory diseases. A population-based cohort study found that people prescribed oral glucocorticoids for a period of at least 15 days had a relative risk of acquiring various bacterial, viral or fungal infections that was 2- to 6-fold higher than equivalent comparators who were not exposed to glucocorticoids (Fardet et al., 2016). Another population-based cohort study of people prescribed short courses of oral corticosteroids (median 6 days) found that in the 30 days following drug initiation there was a 5-fold increase in rates of hospitalisation for sepsis (Waljee et al., 2017).

126. Information relevant to the dose levels of GMO that may cause immunosuppression is protected as CCI. As the bodyweight of children or pets is lower than adults, doses of GMO lower

[12] Relevant information from animal studies characterising the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

than the dose that causes immunosuppression in adults could cause immunosuppression in the gastrointestinal tract of children or pets.

127. As discussed in Chapter 1, Section 4, large doses of VIP cause high-volume secretion of water and electrolytes into the intestines, manifesting in severe watery diarrhea. Patients with prolonged secretory diarrhea caused by VIP often require hospitalisation for rehydration and electrolyte replacement. However, a short bout of secretory diarrhea is unlikely to require hospitalisation in people or pets who are otherwise healthy.

128. It is noted that secretory diarrhea could have an incidental effect of quickly clearing the GMO and secreted VIP out of the gastrointestinal tract.

129. There is uncertainty regarding the dose levels of GMO or secreted VIP that could cause secretory diarrhea. Based on the human studies discussed in Chapter 1, Section 4.1.1., VIP administered by infusion causes moderate-to-severe diarrhea at doses approximately twofold higher than doses used for therapeutic immunosuppression. It is not known whether this relationship between doses causing immunosuppression and diarrhea would be the same when VIP is delivered into the gastrointestinal tract rather than systemically.

Conclusion

130. The potential for accidental ingestion of GMO doses by people or pets resulting in ill health is not identified as a risk that could be greater than negligible. The main reasons are that the proposed packaging and controls minimise the potential for people other than trial participants or pets to ingest GMO doses, and the GMO is not expected to colonise human or animal guts, so any adverse effect would be transitory. Therefore, this risk scenario does not warrant further detailed assessment.

2.6 Risk scenario 2

Risk source	GMO secreting VIP
Causal pathway	Clinical trial participants shed GMO in stool, vomit and/or saliva ⬇ People other than trial participants are exposed to the GMO ⬇ GMO enters gut and secretes VIP
Potential harm	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications

Risk source

131. The source of potential harm for this postulated risk scenario is the GMO, which secretes VIP.

Causal Pathway

Shedding of GMO

132. The GMO would be administered to trial participants orally, and therefore GMO could be present in saliva, vomit and stools.

133. As discussed in Chapter 1, Section 3, *L. brevis* is not adapted to live in human or animal hosts, but is capable of surviving gut transit. Therefore, a large proportion of the GMO ingested by trial participants would be shed in stool as live bacteria.[13]

134. As discussed in Risk Scenario 1, the GMO could cause diarrhea in the healthy trial participants enrolled in parts A and B of the clinical trial, so these participants could have frequent bowel movements that each shed GMO. Trial participants enrolled in part C of the proposed clinical trial would have active, mild to moderate ulcerative colitis, which causes patients to have up to six bowel movements per day (Tripathi and Feuerstein, 2019). In addition, over 40% of patients with active ulcerative colitis are reported to suffer from bowel incontinence, although this figure includes patients with severe disease (Newton et al., 2019; Kamal et al., 2021).

135. Live GMO could be shed in vomit during the period that the GMO is present in the stomach. Vomiting is highly unlikely to occur in the healthy trial participants enrolled in parts A and B of the proposed clinical 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). Therefore, vomiting could occur in trial participants enrolled in part C of the trial who have ulcerative colitis.

136. Live GMO could also be shed in saliva under some circumstances[14] or as a result of reflux.

Exposure to the GMO

137. People other than trial participants could be exposed to live GMO shed by trial participants.

138. The applicant states that the proposed clinical trial will only enrol trial participants who agree to abstain from unprotected anal sex. During the period of the clinical trial, no medical professional would accept a faecal transplant donation from a trial participant. Therefore, these exposure pathways are implausible.

139. During the clinical trial, participants could contaminate their hands with shed GMO. This could happen during normal toilet use, collection of stool samples, or clean-up after incidents of vomiting or bowel incontinence. If trial participants do not thoroughly decontaminate their hands, the GMO could be transmitted to other people. The applicant proposes to instruct clinical trial participants in appropriate hygiene measures, such as hand washing after using the toilet. This measure would reduce the likelihood of exposure to the GMO.

140. Household contacts of trial participants could be directly exposed to shed GMO, for example, when cleaning bathrooms or when laundering clothing or linens soiled by an incident of bowel incontinence. If saliva contains GMO, close contacts of trial participants could be exposed to the GMO through kissing or shared utensils. A trial participant engaged in food preparation could transfer GMO to the food via tasting, sneezing or coughing, leading to exposure of people who consume the food.

Secretion of VIP

141. As discussed in Risk Scenario 1, if GMO enters the gastrointestinal tract of people, it will secrete VIP there. However, the GMO is not expected to colonise human or animal gastrointestinal tracts, so secretion of VIP in the gut would be transitory.

[13] Relevant information from animal studies characterising the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

[14] Relevant information about the dosage form is protected as CCI.

Potential harm

142. The potential harms for this risk scenario are the same as the potential harms described in detail in Risk Scenario 1. The GMO secreting VIP could cause immunosuppression in the gastrointestinal tract leading to increased rates of infections and/or secretory diarrhea and associated health complications.

143. However, based on information about GMO dose levels that is protected as CCI, it is highly unlikely that people other than trial participants could accidentally ingest a dose of GMO high enough to cause adverse effects through exposure to the stool, vomit or saliva of a trial participant. Therefore, although people other than trial participants could be exposed to the GMO, the potential exposure doses are considered too low to cause adverse health effects.

Conclusion

144. The potential for exposure to GMO shed by trial participants resulting in ill health in other people is not identified as a risk that could be greater than negligible. The main reasons are that people would be exposed to GMO at doses too low to cause adverse health effects, and that the GMO is not expected to colonise the human gut, so any adverse effect would be transitory. Therefore, this risk scenario does not warrant further detailed assessment.

2.7 Risk scenario 3

145. Risk Scenario 3 considers the potential for spread of GMO in the environment leading to consumption of GMO in plant material and resulting in ill health in humans or animals. As Risk Scenario 3 is considered to be a substantive risk, a risk characterisation was conducted as detailed in Section 3.

2.8 Risk scenario 4

Risk source	GMO containing VIP gene
Causal pathway	GMO is present in gut of clinical trial participants ⬇ VIP gene is horizontally transferred to gut bacteria ⬇ Novel GM gut bacteria secreting VIP persist in clinical trial participants and spread to other people
Potential harm	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications

Risk source

146. The source of potential harm for this postulated risk scenario is the GMO, which contains an introduced gene encoding VIP.

Causal Pathway

GMO presence in gut

147. During the clinical trial, trial participants will ingest GMO doses once per day. Therefore, the GMO is expected to be present in the gastrointestinal tracts of trial participants over the period of the clinical trial.[15]

Horizontal gene transfer of VIP gene

148. While the GMO is present in the gastrointestinal tracts of clinical trial participants, the introduced VIP gene could be horizontally transferred to bacteria that are normally resident in the gut. Horizontal gene transfer (HGT) in gut bacteria occurs frequently. For example, the rate of gene acquisition in the pangenome of five bacterial species in an individual person was reported as 900 genes per year (Groussin et al., 2021).

149. HGT can occur via three pathways between bacteria: (a) transfer of plasmids via conjugation, (b) transformation of competent bacteria and (c) transduction via bacteriophages.

a) Conjugation is thought to contribute the largest proportion of HGT between bacteria (Huddleston, 2014; Neil et al., 2021). Considering confidential information supplied by the licence applicant[16], HGT of the VIP gene to other bacteria via conjugation is highly unlikely to occur in the proposed release.

b) Transformation of bacteria can occur when the recipient takes up a DNA fragment (Huddleston, 2014). This mechanism depends on several steps: DNA of the donor must be released into the gut, be dispersed and persist. In the gut, mechanical and enzymatic activity would fragment any free DNA. However, if bacteria around DNA fragments are in a competent state, then they may take up these DNA fragments. Competence can be brought about by various environmental stimuli, such as starvation. After take-up by the recipient, if highly homologous DNA regions are present between the DNA fragment and the DNA of the recipient bacteria, homologous recombination can occur. This would lead to the gene fragment being incorporated into the DNA of the recipient. The requirement of homology would restrict HGT of the VIP gene via transformation to a small number of bacterial species[17] which would limit the likelihood of this pathway.

c) Bacteriophages are viruses that infect bacteria. Transduction via bacteriophages can occur when the genome of a bacteriophage is incorporated into the genome of the DNA donor bacteria as a prophage. After receiving an environmental stimulus, the prophage is activated and excises from the host genome. This excision step is highly imprecise, and the phage may take part of its host's genome with it. Upon infection of the next host cell, this DNA is released into the new host cell and may integrate into the new host's genome (Huddleston, 2014). However, the applicant states that the genome of the GMO does not contain any inducible prophage sequences. Therefore, HGT of the VIP gene to other bacteria via bacteriophages is highly unlikely to occur.

[15] Relevant information from animal studies characterising the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

[16] Relevant information about the genome of the GMO is protected as CCI.

[17] Relevant information about the genetic modifications is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

150. The small number of clinical trial participants and the limited duration of treatment further reduce the likelihood of HGT occurring. Therefore, HGT of VIP from the GMO to a gut bacterium is considered highly unlikely.

151. If HGT occurred, successful expression of VIP could only occur if the entire gene sequence were available after HGT. Since VIP is a small peptide, the entire gene sequence may be transferred within an HGT event.

152. As discussed in Chapter 1, Section 4, the GMO LIV001 has been designed for high expression of VIP.[18] Therefore, if the VIP gene cassette was horizontally transferred to a gut bacterium, the novel GM bacterium would be expected to secrete VIP at a lower level than LIV001.

Novel GM gut bacteria persist and spread

153. A novel GM gut bacterium that acquired the VIP gene by HGT could multiply and persist in clinical trial participants if the VIP gene provides a selective advantage. In healthy trial participants, secretion of VIP is not expected to increase bacterial fitness. However, in trial participants with ulcerative colitis, bacteria secreting VIP could reduce local gut inflammation. This could provide an advantage because a non-inflamed gut is more hospitable to bacteria than an inflamed gut. In a study of the effects of inflammation on intestinal microbiota, mice with intestinal inflammation induced by different methods had colon bacteria concentrations reduced by 30-75% compared to healthy animals (Lupp et al., 2007). However, the selective advantage for a bacterium that secretes VIP would be limited, because the advantage of reduced local inflammation would be shared by neighbouring bacteria even if they do not secrete VIP.

154. If novel GM gut bacteria persisted in clinical trial participants, they could be transmitted to other people via the pathways described in Risk Scenario 2. A recent study of person-to-person transmission of gut bacteria found 12% median strain sharing between cohabiting individuals, 8% median strain sharing between individuals residing in the same village, and 0% median strain sharing between individuals residing in different villages of the same population (Valles-Colomer et al., 2023). Therefore, transmission of a persistent GM gut bacteria strain from a trial participant to close contacts, resulting in gut colonisation, is plausible. However, it is highly unlikely that the novel GM gut bacteria would spread widely within a population, both based on this study and because no selective advantage is anticipated in healthy humans.

Potential harm

155. The potential harms for this risk scenario are the same as the potential harms described in detail in Risk Scenario 1. Novel GM gut bacteria secreting VIP could cause immunosuppression in the gastrointestinal tract leading to increased rates of infections and/or secretory diarrhea and associated health complications.

156. If novel GM gut bacteria secreting VIP caused obvious adverse health effects, the bacteria could be treated with antibiotics.

Conclusion

157. The potential for horizontal transfer of the VIP gene to gut bacteria resulting in ill health in people other than trial participants is not identified as a risk that could be greater than negligible. The main reasons are that the small scale of the clinical trial minimises the likelihood of HGT events,

[18] Relevant information about the genetic modifications is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

and that GM gut bacteria could be treated with antibiotics. Therefore, this risk scenario does not warrant further detailed assessment.

2.9 Risk scenario 5

Risk source	GMO containing VIP gene
Causal pathway	Clinical trial participants shed GMO in stool, which enters sewage ⬇ VIP gene is horizontally transferred to bacteria in sewage ⬇ Novel GM bacteria survive sewage treatment and are released in treated effluent or biosolids ⬇ Humans or animals are exposed to novel GM bacteria secreting VIP
Potential harm	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications

Risk source

158. The source of potential harm for this postulated risk scenario is the GMO, which contains an introduced gene encoding VIP.

Causal Pathway

GMO enters sewage

159. The GMO would be administered to trial participants orally. As discussed in Chapter 1, Section 3, *L. brevis* is not adapted to live in human or animal hosts, but is capable of surviving gut transit. Therefore, a large proportion of the GMO ingested by trial participants would be shed in stool as live bacteria.[19] In most cases, trial participants would excrete stool containing GMO into toilets connected to an urban sewage system.

HGT of VIP gene to bacteria in sewage

160. The GMO would enter sewage and mix with other bacteria there. This could provide an opportunity for the VIP gene to be horizontally transferred from the GMO to another bacterium. As discussed in Risk Scenario 4, the small scale of the clinical trial minimises the likelihood of HGT of the VIP gene from the GMO to a bacterium in the gut. Similarly, the small scale of the clinical trial minimises the likelihood of HGT of the VIP gene from the GMO to a bacterium in sewage.

Novel GM bacteria survive sewage treatment and are released

161. Most bacteria in sewage are killed by standard wastewater treatment. Therefore, even if a novel GM bacterium was generated by HGT in sewage, it would probably not survive. However, some bacteria can survive wastewater treatment and be released into the environment. For example, in a recent study of twelve wastewater treatment plants in Western Australia, the spore-forming bacterium *Clostridium difficile* was found in 91% of untreated sewage influent, 48% of treated

[19] Relevant information from animal studies characterising the GMO is protected as CCI.

effluent intended for release into natural water bodies or irrigation use, and 94% of treated biosolids intended for application to agricultural land (Chisholm et al., 2023).

Exposure to GM bacteria secreting VIP

162. If a novel GM bacterium was released into environments such as natural water bodies or agricultural land, it could multiply, and people or animals could be exposed to the GM bacteria through food or water. However, as the GM trait of secreting VIP is not expected to increase bacterial fitness, there is no reason for the GM trait to become fixed in the population of the bacterial species. Therefore, people or animals could only be exposed to very low levels of the novel GM bacteria.

Potential harm

163. The potential harms for this risk scenario are the same as the potential harms described in detail in Risk Scenario 1. Novel GM bacteria secreting VIP could cause immunosuppression leading to increased rates of infections and/or secretory diarrhea and associated health complications.

Conclusion

164. The potential for horizontal transfer of the VIP gene to bacteria in sewage resulting in ill health in humans or animals is not identified as a risk that could be greater than negligible. The main reasons are that the small scale of the clinical trial minimises the likelihood of HGT events, and that as VIP secretion is not expected to increase bacterial fitness, the GM trait would not become fixed in a bacterial population. Therefore, this risk scenario does not warrant further detailed assessment.

Section 3 Risk characterisation

165. Five risk scenarios were postulated and evaluated, as summarised in Table 2. The third risk scenario was identified as posing a substantive risk which warrants further assessment. This section provides more detail on the characterisation of this risk.

166. Risk characterisation involves a likelihood assessment, a consequence assessment, a risk estimate, and a decision on whether risk treatment is required. See the Risk Analysis Framework (OGTR, 2013) for further information about the OGTR's approach to conducting risk analysis.

3.1 Risk scenario 3

Risk source	GMO secreting VIP
Causal pathway	1a. GMO is released into the outdoor environment via loss of GMO doses OR 1b. GMO is released into the outdoor environment via shedding of live GMO ⬇ 2. GMO establishes on plant substrates ⬇ 3. GMO spreads widely in the environment ⬇ 4a. People or animals consume non-fermented food plants containing the GMO and/or secreted VIP at levels that cause adverse health effects OR 4b. People or animals consume fermented food plant products containing the GMO and/or secreted VIP at levels that cause adverse health effects
Potential harm	Suppression of immune system in gastrointestinal tract, increasing susceptibility to pathogen infection and development of disease AND/OR Secretory diarrhea and associated health complications

Risk source

167. The source of potential harm for this postulated risk scenario is the GMO, which secretes VIP.

3.2 Likelihood assessment

168. A likelihood assessment determines the chance that harm may occur, ranging from highly unlikely to highly likely. The likelihood assessment for the causal pathway for Risk Scenario 3 is presented below. The causal pathway is divided into numbered steps. The likelihood of each step is assessed, followed by assessment of the cumulative likelihood of the causal pathway.

Step 1a – GMO is released into the outdoor environment via loss of GMO doses

169. During the proposed clinical trial, live GMO could be released into the outdoor environment via loss of GMO doses. Some potential pathways for loss of GMO doses during transport, storage and self-administration by trial participants are described below.

170. In parts B and C of the proposed clinical trial, GMO doses would be dispensed to trial participants for self-administration at home. Some trial participants may drop out of the proposed clinical trial. In a similar clinical trial testing an eight-week oral probiotic treatment for irritable bowel symptom, 20% of participants dropped out of the trial, including 9% who dropped out in the first two weeks (Stevenson et al., 2014). Part B of the proposed clinical trial would involve 12 participants receiving GMO treatment over a period of two weeks, and Part C of the proposed clinical trial would involve about 10 participants receiving GMO treatment over a period of eight weeks (Chapter 1, Section 2). Based on the withdrawal rates in the cited study and the intended enrolment numbers in parts B and C of the proposed clinical trial, a small number of participants could drop out of the

proposed clinical trial with unused GMO in their possession. Participants who drop out of the clinical trial could discard unused doses of the GMO into domestic waste.

171. Trial participants could also accidentally discard some GMO during self-administration of doses.[20]

172. Trial participants may accidentally lose containers of the GMO during transport or storage. In a large survey of adherence to oral diabetes medication, 0.25% of respondents reported losing their medicine in the prior 4 weeks (Vietri et al., 2016). This suggests, considering the small scale of the clinical trial, that loss of a GMO container is unlikely to occur. If a container of GMO was lost, and found by another person, it would probably be discarded into waste.

173. If the GMO is discarded into household waste by any of the pathways above, any breach of the GMO packaging would release the GMO into the waste. *L. brevis* grows well on food waste (Probst et al., 2013), which is 30-40% of Australian household waste (Arcadis, 2019), so the GMO could multiply and spread in domestic waste once released from packaging.

174. After delivery to an Australian landfill, waste is covered with a daily cover such as 15 cm of soil at the end of each day, prior to final capping when the landfill cell is full (Environmental Guidelines: Solid waste landfills). It is possible that waste containing the GMO could spread outside the area to be covered before the daily covering is applied, via wind, water runoff or scavenger activity. For example, urban seagulls in Australia regularly feed at landfill sites (Stewart et al., 2020), and could subsequently excrete GMO into the outdoor environment. However, almost all waste that is delivered to a landfill remains at the landfill.

175. The likelihood of step 1a is assessed as **highly unlikely**, due to the improbability of the GMO being moved from a landfill into the wider environment. In addition, the number of times that GMO doses could enter landfill waste is limited.

Step 1b – GMO is released into the outdoor environment via shedding of live GMO

176. During the proposed clinical trial, live GMO could be released into the outdoor environment via shedding of the GMO in stool, vomit or saliva. Some examples of plausible pathways for release of shed GMO are described below.

177. As discussed in Risk Scenario 2, a large proportion of the GMO ingested by trial participants would be shed in stool as live bacteria. In most cases, trial participants would excrete stool containing GMO into standard toilets connected to an urban sewage system. In 2021, approximately 54% of urban sewage in Australia underwent tertiary wastewater treatment (Bureau of Meteorology, 2023). Tertiary wastewater treatment involves disinfection and is expected to kill the GMOs, which are not spore-forming bacteria. Almost all Australian urban sewage effluent that is not treated to tertiary level is discharged into the ocean. As *L. brevis* is adapted to live on terrestrial plant substrates, the GMO is not expected to survive in the ocean. 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 4/9/2023). In this case, there is uncertainty whether excreted GMO would survive wastewater treatment. It is highly unlikely that any clinical trial site would be located in an inland town and unlikely that any trial participant would live, work or stay in an inland town during the period of the proposed clinical trial. Therefore, release of live GMO into the outdoor environment via insufficiently treated urban sewage effluent is considered unlikely.

[20] Relevant information about dosage form and packaging of the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

178. Urban sewage treated at a wastewater treatment plant produces biosolids as well as effluent. In 2021, about 83% of biosolids produced in Australia were reused, including about 73% that were applied as fertiliser to agricultural land (Australian Biosolids Statistics website, accessed 4/9/2023). 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). For parts A and B of the clinical trial, the proposed clinical trial facility is in Melbourne (Chapter 1, Section 2.3.3). Melbourne Water processes over 90% of sewage generated in Melbourne, including sewage from the suburb where the clinical trial facility is located, and treats all biosolids for reuse to grade A level (referred to as T1 grade in Victoria) (Melbourne Water website, accessed 7/9/2023). The smaller wastewater treatment plants in Melbourne would probably also treat any biosolids for reuse to T1 grade, as the outlets for lower grade products are very limited in Victoria (Yang et al., 2018). Thus, GMO shed in stool in Melbourne is not expected to survive in treated biosolids. Part C of the clinical trial could use multiple clinical trial facilities across Australia. GMO shed in stool during part C of the trial could survive in grade B level biosolids and be applied to agricultural land. Therefore, release of live GMO into the outdoor environment via biosolids is considered likely.

179. Untreated sewage is sometimes released from urban sewage systems due to overflow events, particularly during wet weather. In 2020-2021, the volume of wastewater losses and spills in Australia was approximately 3% of total wastewater collected (Bureau of Meteorology, 2023). 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. Due to the small size of the proposed clinical trial, release of live GMO into the outdoor environment via overflow of raw sewage is considered unlikely.

180. Participants in Part A of the clinical trial would remain at a clinical trial site for three days after their only dose of GMO, so would only use standard toilets connected to urban sewage systems while shedding GMO in stool. Participants in parts B and C of the clinical trial may use other types of toilets, such as composting toilets or septic tank systems, at their homes and workplaces during the trial. The GMO could survive in composting or septic tank systems, and if the contents are subsequently dispersed outside, this could release GMO into the outdoor environment. In 2016, 7% of Australian households were not connected to urban sewage systems (Vaughan et al., 2017). This includes many households in cities, for instance, in Sydney in 2015-16, 4% of the population connected to urban water services were not connected to urban sewage systems. Therefore, it is considered likely that at least one of the 22 trial participants administered the GMO in parts B and C will use a composting toilet or septic tank system, resulting in the release of live GMO into the outdoor environment.

181. If trial participants in part B or C of the clinical trial engage in outdoor activities, such as bushwalking or camping, they may need to pass stools in locations where there are no toilets. This would release GMO into the outdoor environment. There is uncertainty about the likelihood of this pathway, but it is estimated as highly unlikely due to the small number of trial participants and the limited duration of the treatment.

182. As discussed in Risk Scenario 2, trial participants in part C of the trial could suffer from bowel incontinence. If the trial participants use incontinence products such as pads, soiled incontinence products containing the GMO would probably be discarded into waste that is destined for landfill. However, as discussed in step 1a, it is highly unlikely that the GMO would escape from a landfill into the wider environment.

183. If pets accidentally ingest doses of the GMO, the stools of the pets would contain GMO. In most cases, the pets would subsequently defecate outside, releasing GMO into the outdoor environment.

As discussed in Risk Scenario 1, it is highly unlikely that pets would ingest and subsequently release the GMO.

184. Clinical trial participants could vomit outside during the period of the proposed clinical trial.[21] Vomiting would be very rare in healthy trial participants. However, as discussed in Risk Scenario 2, vomiting is a symptom of patients with ulcerative colitis, reported to occur in about 25% of patients (including patients with severe disease). Therefore, some trial participants in part C of the clinical trial may be subject to vomiting. If trial participants are outside when they feel a need to vomit, they are expected to vomit outside. A large US activity survey found that people spend, on average, only 7.6% of their time outside (Klepeis et al., 2001). Therefore, the likelihood for this pathway is estimated as unlikely.

185. As discussed in Risk Scenario 2, the GMO could be shed in saliva under some circumstances[22]. Trial participants in parts B and C of the clinical trial would self-administer GMO doses at home. If their saliva contains GMO, food leftovers could be contaminated with the GMO. If food waste is placed in compost, the GMO could multiply there, as *L. brevis* grows well on food waste (Probst et al., 2013). The GMO would later be released into the outdoor environment in compost. There is uncertainty about the likelihood of this pathway, but it is estimated as highly unlikely due to the limited number of trial participants and the restricted circumstances in which the GMO could be shed in saliva.

186. As discussed in Risk Scenario 2, clinical trial participants could contaminate their hands with shed GMO. If they do not thoroughly decontaminate their hands, and subsequently engage in outdoor work such as gardening, this could release GMO into the outdoor environment. Alternatively, if trial participants have a home greywater irrigation system, water used for washing hands contaminated with GMO could enter the greywater system and be released into the outdoor environment. However, the amounts of viable GMO released via these pathways are expected to be minimal, so the likelihood is estimated as highly unlikely.

187. The likelihood of step 1b is the likelihood that at least one of the pathways that release GMO into the outdoor environment will occur. The pathways described above are independent events. The probability that at least one of a group of independent events will occur is the union of the probability of each event, which is higher than the probability of any single event (Pishro-Nik, 2014). The events include two likely pathways and three unlikely pathways for release of the GMO. Highly unlikely pathways do not significantly contribute to the likelihood of the step. Therefore, the likelihood of step 1b is assessed as **highly likely**.

Step 2 – GMO establishes on plant substrates

188. If the GMO is released into the outdoor environment, it could potentially establish an ongoing population, depending on the site of release. The release pathways described in steps 1a and 1b could release the GMO on a vegetated area of land, on a non-vegetated area of land, or into an inland water body.

189. As discussed in Chapter 1, Section 3.2, *L. brevis* is ubiquitous on plants and decomposing plant material. It grows on a wide range of plant species and in climates ranging from subtropical to cold and arid.[23] Therefore, if the GMO is released on a vegetated area, it is considered likely to establish.

[21] Relevant information from animal studies characterising the GMO is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

[22] Relevant information about the dosage form and details of administration of the GMO is protected as CCI.

[23] Relevant information about the parental strain of the GMO is protected as CCI.

190. If the GMO is released on a non-vegetated area, such as a paved area or bare ground with no decomposing plant material, it would have no food source, unless it is rapidly washed to a vegetated area. Therefore, it is highly unlikely to establish.

191. *L. brevis* is not adapted to live in water. If the GMO is released in an inland water body, it would not survive unless it is transported to waterside vegetation, or further afield by flooding or use of irrigation water. Therefore, it is highly unlikely to establish.

192. The overall likelihood that GMO released into the outdoor environment establishes on plant substrates is assessed as **unlikely**.

Step 3 – GMO spreads widely in the environment

193. Once the GMO is established in a vegetated area, it could spread in the environment by a number of mechanisms. For instance, GMOs growing on plants could be consumed by animals or birds, survive gut transit, and be excreted at new locations. GMOs growing on plants could be transported by human activity, e.g. during plant harvesting or mowing. GMOs growing on rotting plant material could be dispersed when used as compost or on human or animal feet or vehicle wheels. GMOs growing on any substrate could also be transported by wind or by water.

194. Globally, *L. brevis* is ubiquitous in the environment (Rychen et al., 2016). In the long term, the GMO could spread widely in the environment if it has a selective advantage over non-GM strains of *L. brevis* in Australia. This could be a broad selective advantage, or a selective advantage in some environmental niches. Spreading widely in the environment is taken to mean being established at many locations in Australia.

195. The introduced VIP gene is only known to have a biological function in animals, so it is not expected to provide any selective advantage in a bacterium growing on plant substrates.[24] The genetic modifications would not significantly increase or decrease metabolic burden, considering that *L. brevis* has over 2000 genes (Feyereisen et al., 2019). Overall, the effects of the genetic modifications are expected to have a neutral or slightly deleterious effect on fitness. Therefore, the GMO is not expected to have a selective advantage over its parental strain.

196. However, it is also necessary to consider whether the parental strain of the GMO has a selective advantage over non-GM strains of *L. brevis*. The genomes of *L. brevis* strains differ from each other by hundreds of genes (Feyereisen et al., 2019), so the effect of strain on fitness could be much larger than the effect of the genetic modifications.

197. There is no data regarding the comparative fitness of the parental strain of the GMO and other strains of *L. brevis* in the environment.

198. The likelihood of the GMO spreading widely in the environment is strongly influenced by uncertainty about the competitiveness of the parental strain compared to other *L. brevis* strains in the environment. The likelihood of the GMO spreading widely is assessed as **highly unlikely** (if the GMO has no selective advantage) to **likely** (if the GMO has a selective advantage).

Step 4a – People or animals consume non-fermented food plants containing the GMO and/or secreted VIP at levels that cause adverse health effects

199. If the GMO was able to spread widely in the Australian environment, it could grow on plants that are subsequently eaten by people or animals.

[24] Information about the genetic modifications other than introduction of the VIP gene is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

200. As discussed in Chapter 1, Section 3, *L. brevis* is found at low levels on plant surfaces. In addition, fresh food plants intended for human consumption are typically washed, peeled and/or cooked. These processes would remove or kill almost all microorganisms on the surface of food. Therefore, humans could only be exposed to negligible quantities of the GMO by consuming non-fermented food plants.

201. Animals could consume fresh plant material containing the GMO. Using mice as an example of animals that eat plant material in the Australian environment, a mouse eats approximately 5.3 g/day (Hambly and Speakman, 2005). Plant material is reported to contain up to 2000 CFU/g of *Lactobacillus* species (Chapter 1, Section 3.2). Thus, a mouse could consume up to 10^4 CFU/day of *Lactobacillus* bacteria, although only part of this could be the GMO. Based on information about GMO dose levels that is protected as CCI, levels of the GMO that cause adverse health effects are far higher than the levels that mice could consume. As shown by this example, animals consuming non-fermented plant material containing the GMO would not be exposed to enough GMO to cause adverse health effects.

202. The likelihood of people or animals consuming non-fermented plant material containing the GMO and/or secreted VIP at levels that cause adverse health effects is assessed as **highly unlikely**.

Step 4b – People or animals consume fermented food plant products containing the GMO and/or secreted VIP at levels that cause adverse health effects

203. If the GMO spread widely in the environment, the GMO could be present on plant material that is subsequently used to make fermented food or feed products.

204. A large American survey reported that 17% of adults eat fermented plant foods regularly, i.e. at least three times per week (Taylor et al., 2020). Some fermented foods are made from washed raw food by adding starter culture, but others are naturally fermented using microorganisms that are present in the raw food (Department of Health, 2023). Naturally fermented foods could contain the GMO.

205. In 2020-21, Australia produced 3.8 million tonnes of silage as feed for livestock (Australian Bureau of Statistics, 2022). Plant materials used for silage are not washed to remove microorganisms prior to fermentation, so silage could contain the GMO. If silage is dispersed into paddocks for livestock, wildlife could incidentally feed on the silage. However, silage is unlikely to be an ongoing major dietary component for free-ranging wildlife.

206. If the GMO was present in a fermented product, the GMO could multiply to high levels, as *L. brevis* is abundant in vegetable and cereal fermentations (Chapter 1, Section 3). The paragraphs below estimate the levels of GMO that could be consumed by people in kimchi or by livestock in maize silage, which are examples of fermented food or feed with well-characterised microbiology.

207. A study of microbial population dynamics in a radish kimchi reported that after two weeks fermentation, the concentration of *L. brevis* was 2×10^8 CFU/mL (Ahn et al., 2015). If a person eats 50 mL of kimchi per day, the dose of *L. brevis* consumed would be about 1×10^{10} CFU per day.[25]

208. A study of the bacterial community in silage reported that after 8 weeks ensiling, the concentration of lactic acid bacteria in maize silage was 7.4×10^7 CFU/g (Li and Nishino, 2013). Given that dairy cows can be fed about 40 kg/day of silage when pasture availability is limited (Kaiser et al., 2004), and assuming the average weight of a cow is 650 kg (Pauls Dairy website), this equates to

[25] Relevant information from animal studies characterising the GMO is protected as CCI.

consuming about 4.6 x 10^9 lactic acid bacteria per kg body weight per day. The study found that *L. brevis* was the most abundant lactic acid bacteria in maize silage (Li and Nishino, 2013).[26]

209. Another point to consider is that if the GMO is present during fermentation of food or feed, it is designed to continuously secrete VIP, so it would presumably continuously secrete VIP over the weeks of fermentation. As discussed in Chapter 1, Section 4, endogenous human VIP is rapidly degraded by proteases, but the GMO secretes a stabilised synthetic VIP analogue. There is uncertainty regarding the half-life of the stabilised VIP in fermenting food or feed. If the synthetic VIP is sufficiently stable in a ferment environment, the synthetic VIP could accumulate to a biologically relevant dose in the fermenting food or feed. In this case, people consuming the fermented food or animals consuming the fermented feed could ingest a substantial dose of free VIP in addition to ingesting the GMO that secretes VIP.

210. It is noted that, as discussed in Risk Scenario 1, there is some uncertainty about the dose levels of the GMO that could cause secretory diarrhea. There is also uncertainty about whether some sub-populations could be more vulnerable to adverse effects from VIP.

211. Based on the information above, the likelihood of people or animals consuming fermented plant material containing the GMO and/or secreted VIP at levels that cause adverse health effects is conservatively assessed as **highly likely** for people and livestock.

Overall likelihood assessment

212. The overall likelihood assessment is the cumulative likelihood of the individual steps in the causal pathway. As step 1a is far less likely than the alternative step 1b, and step 4a is far less likely than the alternative step 4b, only the pathway through steps 1b and 4b will be considered. The probability of all four events in the causal pathway occurring is the product of the probability of each event (Pishro-Nik, 2014). The likelihoods of the individual steps in the causal pathway are highly likely (step 1b), unlikely (step 2), highly unlikely to likely (step 3) and highly likely (step 4b). Therefore, the overall likelihood is assessed as **highly unlikely to unlikely**.

3.3 Consequence assessment

213. A consequence assessment determines the degree of seriousness of harm to people or the environment, ranging from marginal to major. The potential harms for this risk scenario are either that VIP could cause increased rates of infections due to immunosuppression, or it could cause secretory diarrhea and associated health complications. Harms could occur in people or in livestock. The consequence of each type of harm is considered separately below, followed by an overall consequence assessment.

Immunosuppression in people

214. As discussed in Chapter 1, Section 4, VIP is a signalling molecule with an anti-inflammatory effect. The intended therapeutic effect in the clinical trial is to suppress inflammation in the gastrointestinal tract, which has many receptors for VIP (Iwasaki et al., 2019). Exposure of people to the GMO that secretes VIP or directly to VIP via the diet could suppress the local immune system in the gastrointestinal tract. This could increase susceptibility to infections by pathogens whose portal of entry is the gastrointestinal tract. As discussed in Risk Scenario 1, this harm is similar to the elevated rate of infections observed in people prescribed corticosteroids to treat inflammatory diseases.

215. If a person is locally immunosuppressed for a period due to consumption of the GMO and/or VIP in fermented food, there would be no obvious symptoms, and the person would take no action. If

[26] Relevant information from animal studies characterising the GMO is protected as CCI.

the person acquires an infection as the result of immunosuppression, the person would have symptoms and would seek treatment for the infection if necessary.

216. If VIP causes localised immunosuppression in the gastrointestinal tract of a person, but the person does not acquire any infections requiring treatment, the harm would be **marginal** (minimal or no increase in illness/injury to people). If the person acquires an infection that they would not have acquired if immunocompetent, and the infection requires treatment such as antibiotics, the harm would be **minor** (minor increase in illness/injury to people that is readily treatable). If the person acquires an infection that they would not have acquired if immunocompetent, and the infection requires treatment in hospital, the harm would be **intermediate** (significant increase in illness/injury to people that requires specialised treatment).

217. The consequence assessment of immunosuppression in people is **marginal to intermediate** harm to health.

Secretory diarrhea in people

218. As discussed in Chapter 1, Section 4, high doses of VIP can cause severe secretory diarrhea. If the diarrhea continues for multiple days, the patient may need hospitalisation for dehydration, complications related to electrolyte deficiency, and/or shock.

219. If a person develops secretory diarrhea due to consumption of the GMO and/or VIP in a fermented food, they may associate the diarrhea with the fermented food and stop consuming it. This would halt the illness.

220. If VIP causes a short bout of secretory diarrhea in a person, that does not require medical treatment, the harm would be **marginal**. If the person has secretory diarrhea for an extended period, and requires hospitalisation, the harm would be **intermediate**.

221. The consequence assessment of secretory diarrhea in people is **marginal to intermediate** harm to health.

Immunosuppression in livestock

222. As discussed in Chapter 1, Section 4, the immunomodulatory function of VIP is conserved in mammals. Therefore, consumption of GMO secreting VIP and/or direct consumption of VIP in fermented feed could suppress the local immune system in the gastrointestinal tract of livestock. This could lead to elevated rates of infections, in the same way that this harm could occur in people.

223. Livestock are valued animals in the agricultural environment. Therefore, death of livestock is considered to be a harm to the environment. However, it is considered to be a reversible harm to the environment, as livestock can be replaced from other sources.

224. If VIP causes local immunosuppression in the gastrointestinal tract of livestock, and the animals acquire infections that they would not have acquired if immunocompetent, but the infections are self-resolving or easily treated by a vet, the harm would be **marginal** (minimal or no increase in harm to desirable components of the environment). If some animals acquire serious infections that they would not have acquired if immunocompetent, and they die of illness or are euthanised, the harm would be **minor** (minor increase in damage to desirable components of the environment that is reversible and limited in time and space or numbers affected).

225. The consequence assessment of immunosuppression in livestock is **marginal to minor** harm to the environment.

Secretory diarrhea in livestock

226. VIP may be able to cause severe secretory diarrhea in livestock. Although ruminant livestock have very different stomachs from humans, their intestines are similar, and VIP causes secretory diarrhea in humans by stimulating water and anion secretion into the intestines (Iwasaki et al., 2019).

It is noted that extended periods of secretory diarrhea in humans may require hospitalisation, but hospitalisation is not practical for livestock.

227. If livestock develop secretory diarrhea due to consumption of sufficiently high levels of the GMO and/or VIP in fermented feed, the farmer may notice the symptoms before animals become severely ill. The farmer or a vet may associate the symptoms with the fermented feed and stop use of the fermented feed, which would halt the illness. If a vet prescribed antibiotics, but the animals continued to eat the fermented feed, the antibiotics might be temporarily effective but the secretory diarrhea would return as soon as the course of antibiotics was completed.

228. If VIP causes a short period of secretory diarrhea in livestock, that does not result in death or euthanasia, the harm would be **marginal**. If a small proportion of the livestock in herds die, due to delays in stopping use of the fermented feed, the harm would be **minor**. If a large proportion of livestock in herds die, due to ongoing consumption of the fermented feed, the harm would be **intermediate** (significant increase in damage to desirable components of the environment that is widespread but reversible or of limited severity).

229. The consequence assessment of secretory diarrhea in livestock is **marginal to intermediate** harm to the environment.

3.4 Risk estimate

230. 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, 2013).

231. The likelihood of the GMO being released outdoors, spreading in the environment to be present on food crops, and being consumed by humans or livestock at levels that cause adverse health effects is considered **highly unlikely to unlikely**. The potential consequence to the health of people or to the environment is considered **marginal to intermediate**.

232. The overall risk is therefore estimated as **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation) **to moderate** (risk is of marked concern and will necessitate actions for mitigation that need to be demonstrated as effective).

Section 4 Uncertainty

233. Uncertainty is an intrinsic part 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.

234. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

235. For DIR 197, uncertainty is noted particularly in relation to:

- the survival rate of the GMO after transit through the human gastrointestinal tract
- the ability of the GMO to survive wastewater treatment
- the dose levels of GMO or secreted synthetic VIP that cause local immunosuppression or secretory diarrhea in humans, including in vulnerable populations
- the fitness of the GMO or the parental strain compared to other strains of *L. brevis* in the environment
- the stability of the synthetic VIP in fermenting food or feed.

236. The level of uncertainty in this risk assessment is considered high and impacts on the overall estimate of risk. There is uncertainty regarding some steps of Risk Scenario 3, and after taking the uncertainty into account, this risk is considered to require actions for mitigation. Measures to mitigate this risk are described in Chapter 3, Section 2.

237. Additional information to address uncertainties may be required to assess possible future applications with reduced limits and controls, such as a larger scale clinical trial or the commercial release of the GMO. Chapter 3, Section 4 discusses information that may be required for future releases.

Section 5 Risk evaluation

238. 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.

239. 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.

240. Five risk scenarios were postulated whereby the proposed dealing might give risk to harm to people or the environment.

241. 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 a substantive risk and do not advance in the risk assessment process.

242. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, four of the risk scenarios were not identified as substantive risks. The principal reasons for this include:

- the proposed packaging and controls minimise the potential for people other than trial participants to ingest GMO doses;
- the GMO is not expected to colonise human or animal guts, so any adverse effect would be transitory;
- the small scale of the clinical trial minimises the likelihood of HGT events.

243. Risk Scenario 3 describes a pathway where the GMO is released outdoors, spreads on plant substrates in the environment, is consumed by humans or livestock, and causes adverse health effects. This risk scenario was identified as a substantive risk, so further assessment was required. The likelihood and consequences of the substantive risk 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, 2013). Following risk characterisation, the risk described in Risk Scenario 3 was estimated as posing a **negligible to moderate** risk to human health and safety and the environment.

244. The Risk Analysis Framework describes moderate risk as a risk of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective. Measures to mitigate the identified risk are proposed in Chapter 3, Section 2.

245. 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

246. 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.

247. 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.

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

249. 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

250. The risk assessment of Risk Scenario 3 in Chapter 2 concluded that there is a negligible to moderate risk to people and the environment. The risk involves the GMO being released outdoors, establishing on plant substrates, spreading widely in the environment, being consumed by humans or livestock, and causing adverse health effects.

251. The most effective way to manage this risk by licence conditions is to reduce the likelihood of releasing the GMO outdoors. In Chapter 2, Section 3.2, the cumulative likelihood of step 1b, that GMO is released into the outdoor environment via shedding of live GMO, was assessed as highly likely.

252. The main risk treatment measure imposed to reduce the likelihood of releasing the GMO outdoors is not permitting Part C of the proposed clinical trial to proceed. Parts A and B of the proposed clinical trial involve 180 participant-days of GMO administration, while Part C involves ~560 participant-days (Chapter 1, Section 2.3.3). Therefore, not permitting Part C reduces the scale of the trial by about 75%. This risk treatment measure reduces the likelihood of all five of the plausible pathways for release of the GMO outdoors, due to scale reduction and other reasons, as described below.

253. The first plausible pathway for outdoor release of the GMO is trial participants using toilets connected to sewage systems where some GMOs could survive in treated biosolids. As discussed in Chapter 2, Section 3.2, step 1b, this pathway could occur in many areas of Australia, but is not expected to occur in Melbourne, due to the high level of biosolids treatment in Melbourne wastewater treatment plants. The applicant proposed a clinical trial facility located in Melbourne for Parts A and B of the clinical trial. The licence requires that any clinical trial facility, and the home of any clinical trial participant who is dispensed the GMO for administration at home, must be located in Melbourne. This minimises the likelihood of the GMO that is shed into toilets at a clinical trial

facility or participant homes surviving in treated biosolids. As discussed above, the licence also does not permit part C of the clinical trial to proceed, which decreases the scale of the trial by about 75% in participant-days. This reduces the likelihood that any trial participant will travel outside Melbourne during the clinical trial and shed GMO into toilets where the GMO might survive in biosolids. These risk treatment measures are estimated to reduce the likelihood of this pathway from likely to highly unlikely.

254. The second plausible pathway for outdoor release of the GMO is trial participants using non-standard toilet systems, such as composting toilets or septic tank systems, where some GMOs could survive wastewater treatment. The licence requires that the toilet/s at the home of any clinical trial participant who is dispensed the GMO for administration at home be connected to mains sewage. As discussed above, the licence also does not permit part C of the clinical trial to proceed, which reduces the scale of the trial by about 75% in participant-days. This reduces the likelihood that trial participants will shed GMO into non-standard toilets at venues other than their homes. These risk treatment measures are estimated to reduce the likelihood of this pathway from likely to highly unlikely.

255. The third plausible pathway for outdoor release of live GMO is trial participants using toilets connected to sewage systems where some GMOs could survive in treated effluent. This pathway could occur, for example, if participants use toilets in inland towns (see Chapter 2, Section 3.2, step 1b). As discussed above, the licence requires that any clinical trial facility, and the home of any clinical trial participant who is dispensed the GMO for administration at home, be located in Melbourne. This minimises the likelihood of GMO shed into toilets at a clinical trial facility or participant homes surviving in treated wastewater effluent. As discussed above, the licence also does not permit part C of the clinical trial to proceed, which decreases the scale of the trial by about 75% in participant days. This reduces the likelihood that any trial participant will travel to an inland town during the clinical trial and shed GMO into toilets where the GMO might survive in treated effluent. These risk treatment measures are estimated to reduce the likelihood of this pathway from unlikely to highly unlikely.

256. The fourth plausible pathway for outdoor release of live GMO is overflow of raw sewage containing the GMO, particularly during wet weather (see Chapter 2, Section 3.2, step 1b). As discussed above, the licence does not permit part C of the clinical trial to proceed, which decreases the scale of the trial by about 75% in participant-days. This reduces the likelihood that a storm causing sewage overflow will occur while the GMO is present in sewage pipes. Therefore, this risk treatment measure is estimated to reduce the likelihood of this pathway from unlikely to highly unlikely.

257. The fifth plausible pathway for outdoor release of the GMO is vomiting by trial participants, as vomiting is a symptom of ulcerative colitis (see Chapter 2, Section 3.2, step 1b). Only trial participants in Part C of the proposed trial would have ulcerative colitis, and as discussed above, the licence does not permit part C of the clinical trial to proceed. It is noted that ulcerative colitis is one of the two types of inflammatory bowel disease. The other type of inflammatory bowel disease, Crohn's disease, also causes vomiting (Nag and Romero, 2022). Thus, a licence condition requires the licence holder to ensure that persons diagnosed with inflammatory bowel disease are not enrolled in the clinical trial. This minimises the likelihood that any trial participant would vomit outdoors during the clinical trial. Therefore, this risk treatment measure is estimated to reduce the likelihood of this pathway from unlikely to highly unlikely.

258. The specific risk treatment measures above restrict release of the GMO outdoors, and are considered sufficient to manage the risks associated with Risk Scenario 3.

259. The risk assessment of the remaining four risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed clinical trial with the GMO. These risk scenarios were 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. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

260. 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 moderate. Therefore, to maintain the risk context, licence conditions have been imposed to limit the number of trial participants and 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

261. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by Novotech. Many of these are discussed in the five 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

262. The clinical trial is proposed to enrol approximately 34 trial participants receiving the GMO, with other trial participants receiving placebo. Parts A and B of the clinical trial are proposed to treat 24 participants with the GMO. As Part C of the trial is not permitted to proceed, a licence condition limits the number of clinical trial participants receiving the GMO to a maximum of 28. The licence allows a slightly higher number of trial participants receiving GMO than the applicant proposed for Parts A and B. This is in case a few trial participants withdraw from the trial and need to be replaced. In a similar clinical trial testing an oral probiotic treatment for irritable bowel symptom, 9% of participants withdrew from the trial in the first two weeks (Stevenson et al., 2014).

263. The applicant proposed that participants in Part A of the trial would receive a single dose of GMO, participants in Part B of the trial would receive 14 daily doses of GMO, and participants in Part C of the trial would receive 56 daily doses of GMO. As Part C of the trial is not permitted to proceed, a licence condition requires that the GMO must not be administered to any trial participant for a period longer than 15 days.

264. The applicant has requested a licence for 7 years. A licence condition limits the period when the GMO may be administered under the licence to 7 years from the date of issue.

265. Administration of the GMO is proposed to take place either at clinical trial sites, which are medical facilities, or at the homes of trial participants. GMO doses for home administration would be dispensed to trial participants during clinical trial site visits. To maintain this context, and to facilitate compliance with other licence conditions, the licence does not permit GMO doses to be dispensed to trial participants by means other than clinical trial site visits.

266. The applicant proposed to import the GMO in accordance with IATA shipping classification UN3245 (GMOs that are not classified as category A or B infectious substances), which is a standard protocol for handling and minimising exposure to a GMO. The licence includes this requirement for import or export.

267. The application did not discuss transport of the GMO between clinical trial sites, or between storage facilities and clinical trial sites. However, transport of these types may be necessary during the trial. Licence conditions require that these types of transport comply with minimum

requirements for packaging and labelling the GMO from the Regulator's *Guidelines for Transport, Storage and Disposal of GMOs* for risk group 1 organisms. The term 'storage facilities', as defined in the licence, does not include the homes of trial participants.

268. The applicant proposed that GMO doses would be stored at clinical trial sites in accordance with the Regulator's *Guidelines for Transport, Storage and Disposal of GMOs* for risk group 1 organisms. The licence requires that GMO doses stored at clinical trial sites or storage facilities must be stored in accordance with minimum requirements for packaging and labelling the GMO from the *Guidelines*.

269. The applicant proposed that, at the clinical trial sites, unused GMO or waste containing GMO would be disposed of via the clinical waste stream. This is an acceptable means of disposing of the GMO and is included in the licence. The licence also permits on-site decontamination of the GMO.

270. The applicant proposed to comply with standard measures to clean up any spill of GMOs at a clinical trial site, including using personal protective equipment and a chemical disinfectant. These measures are included in the licence.

271. The applicant proposed that GMO doses would be dispensed to trial participants in a form that is double packaged and ready for administration[27]. Both containers would be labelled "Keep out of the reach of children". This type of packaging was an important reason why Risk Scenario 1 was found to pose negligible risk. Therefore, the licence requires this type of packaging. The containers must also be labelled to indicate that they contain a GMO, which is a standard requirement for packaging when transporting or storing a GMO.

272. As a control, the applicant proposed to track GMO doses that have been dispensed to clinical trial participants for self-administration at home and to destroy any GMO doses that remain unused at the end of the trial. A licence condition requires the licence holder to track all GMO doses dispensed to trial participants and whether they have been used as intended. Another licence condition requires trial participants who self-administer the GMO at home to return all unused GMO doses to a clinical trial site within one week after the final self-administration of the GMO. This includes GMO doses that are unused due to withdrawal of a trial participant from the clinical trial, due to the doses being damaged, spilled or soiled, or due to any other reason. A standard licence condition requires the licence holder to report any contraventions of the licence by a person covered by the licence to the Regulator, so if trial participants do not return unused GMO doses to a clinical trial site, this would be reported to the Regulator. Another standard licence condition requires the licence holder to ensure that all GMO doses or waste containing GMO doses are destroyed before or at the end of the licence.

273. As a control, the applicant proposed to issue spill kits to trial participants who self-administer the GMO at home. These spill kits would be used to clean up any spill of GMO doses[28] that occurs at home, and the contaminated material would be returned in a sealed bag to a clinical trial site for disposal. This measure would minimise the amount of GMO doses being placed in domestic waste so is included in the licence. The licence also requires that the spill kits include means to collect and return any GMO dose that is unsuitable for ingestion because it is spilled, broken, damaged or soiled. The licence holder is required to instruct the trial participants in correct use of the spill kits.

[27] Some information about the GMO packaging is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

[28] Some information about the dosage form of the GMO in the clinical trial is protected as CCI. Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

274. As a control, the applicant proposed to instruct clinical trial participants in appropriate hygiene measures, such as hand washing after using the toilet. The licence requires the licence holder to instruct trial participants in hygiene measures to follow during the clinical trial. The hygiene measures must include: thorough hand washing with soap or hand sanitiser after toilet use or any contact with stool or vomit, cleaning any non-disposable items contaminated with stool or vomit using detergent or cleaning chemicals, discarding any disposable items contaminated with stool or vomit into either a landfill bin or a toilet, and avoiding passing stools in an outdoor location where no toilets are available. These measures reduce the exposure of people to the GMO and the potential for release of the GMO into the environment.

275. As a control, the applicant proposed to only enrol trial participants who agree to abstain from unprotected anal sex during the clinical trial. As discussed in Risk Scenario 1, this activity could expose a person other than a trial participant to the GMO, however, there is no pathway to harm. Therefore, this measure is not included in the licence.

276. In parts A and B of the proposed clinical trial, the application indicates that trial participants would stay at the clinical trial site for three days after the first administration of the GMO. This measure relates to trial participant safety for a first-in-human study and will be reviewed by a HREC. It is not included in the licence.

277. The proposed clinical trial has a range of inclusion and exclusion criteria, which will be reviewed by a HREC. Selected inclusion and exclusion criteria are listed in Chapter 1, Section 2.3, and were considered as part of the risk context. The exclusion criterion barring women who are pregnant or lactating from the clinical trial was an important part of the risk context. The RARMP does not consider risk pathways involving transfer of the GMO or VIP to a foetus, or shedding of the GMO or VIP in breast milk. The inclusion criterion requiring trial participants to be adults was also important to the risk context. The RARMP does not consider potential risks from children conducting dealings with the GMO. Therefore, licence conditions require the licence holder to ensure that pregnant or breastfeeding persons and children are not enrolled in the clinical trial.

278. A standard condition is included in the licence requiring the licence holder to ensure that dealings are conducted to not compromise the health and safety of people and minimise unintentional exposure to the GMO.

279. Another standard condition included in the licence requires the licence holder to inform all people dealing with the GMOs, other than external service providers, of applicable licence conditions. This includes training trial participants to whom licence conditions apply.

280. Further conditions to be implemented in the licence are 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, and staff and trial participant training procedures.

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

281. A number of licence conditions have been imposed to limit and control the clinical trial, based on the above considerations. These include requirements to:

- limit the number of trial participants receiving the GMO to 28;
- limit treatment with the GMO to 15 days;
- only enrol adult trial participants who are not pregnant or breastfeeding;
- dispense GMO doses to trial participants with specified packaging and labelling;
- issue spill kits to trial participants who self-administer the GMO at home;
- require trial participants to return unused doses of the GMO to clinical trial sites;
- instruct trial participants in hygiene measures;

- import the GMO in accordance with IATA shipping classification UN 3245;
- dispose of GMO doses via the clinical waste stream or use other effective decontamination methods.

3.2 Other risk management considerations

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

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

3.2.1 Applicant suitability

283. 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.

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

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

3.2.2 Contingency plans

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

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

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

287. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. As Novotech intends to authorise trial participants to conduct dealings with the GMOs (such as oral self-administration, collection of stool samples and transport), trial participants are persons covered by the licence.

288. Prior to dealings with the GMOs, Novotech 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

289. 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.

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

- identification of the clinical trial sites where the GMOs would be administered or dispensed to trial participants for self-administration
- 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

291. 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.

292. 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.

293. 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

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

- information about the survival rate of the GMO after transit through the human gastrointestinal tract
- information about the ability of the GMO to survive wastewater treatment
- information about the dose levels of GMO or secreted synthetic VIP that could cause either immunosuppression in the gastrointestinal tract or secretory diarrhea in people, including in vulnerable populations
- information about the fitness of the GMO or its parental strain in comparison to other strains of *L. brevis* in the Australian environment
- characterisation of the stability of the synthetic VIP in fermenting food or feed.

Section 5 Conclusions of the RARMP

295. The risk assessment concludes that the proposed clinical trial of the GMO poses negligible to moderate risks to the health and safety of people and to the environment as a result of gene technology. These risks require specific risk treatment measures.

296. The risk management plan concludes that the identified negligible to moderate risks can be managed so as to protect the health and safety of people and the environment by imposing risk treatment measures. Licence conditions are imposed to limit the scale of the trial and to enact the proposed controls to restrict the spread and persistence of the GMO in the environment, as these

were important considerations in establishing the context for assessing the risks. Specific risk treatment measures are imposed in the licence to further restrict release of the GMO into the outdoor environment, to manage the risk of the GMO entering fermented food or feed.

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Appendix A: Summary of submissions from prescribed agencies on the consultation RARMP

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

Submission	Summary of issues raised	Comment
1	• Questions the survival of the modified bacteria in the environment.	Section 3.2 of Chapter 2 of the RARMP considers the potential for the GM bacteria to establish and spread in the environment. This section of the RARMP has been extensively revised to address comments received during consultation.
	• Asks whether the GMO may impact sewer treatment works, evaporation ponds or downstream.	As discussed in Risk Scenario 5 of Chapter 2 of the RARMP, live GMO would enter sewage during the clinical trial. However, the GMO would be diluted to very low levels in wastewater. The diluted GMO would not pose any risk to people or animals exposed to wastewater during or after wastewater treatment.
	• Will waste generated from this trial be handled as medical waste or safe to dispose of into general waste?	As discussed in Section 3.1 of Chapter 3 of the RARMP, unused GMO doses or waste containing GMO doses would be disposed of as clinical waste.
	• Overall, no objections from the City.	Submission has been noted.
2	Unfortunately, the medical centre located in the shire has no capacity to participate in the clinical trial. Further, we have very few patients who suffer from inflammatory bowel disease for whom this research would be of benefit.	Submission has been noted.
3	Appreciates the opportunity to review the Risk Assessment and Risk Management Plan, however, has no comments to provide.	Submission has been noted.
4	Council has no issues.	Submission has been noted.

Submission	Summary of issues raised	Comment
5	While the committee agreed that all risk scenarios were identified, they expressed concern with the individual and collective likelihood assessment of the release pathways of the GMO into the environment based on uncertainty in relation to a number of areas including the growth rate of the GMO, VIP expression levels, persistence of the GMO in the gastrointestinal tract, and the replication and competition potential of the GMO in the environment. The committee recommended the office revise the RARMP to address these concerns and to ensure the consistency of the risk communication.	Further information regarding the persistence of *L. brevis* in the gastrointestinal tract and the prevalence of *L. brevis* in the environment was found in the scientific literature and added to Section 3.2 of Chapter 1 of the RARMP. Additional information about the GMO was requested and provided by the applicant. This information is protected as confidential commercial information (CCI). It is included in the version of the RARMP that contains CCI. The likelihood assessment for spread of GMO into the environment (Section 3.2 of Chapter 2 of the RARMP) was extensively revised to incorporate the additional information, address the residual uncertainty and improve risk communication. Revision of the likelihood assessment resulted in an increased risk estimate (Section 3.4 of Chapter 2 of the RARMP) and changes to risk treatment measures (Section 2 of Chapter 3 of the RARMP).
6	• In step 1b of the likelihood assessment for risk scenario 3, the RARMP should include information below relevant to assessing the likelihood of GMO entering the environment through faeces. - All wastewater plant treatments will not kill all bacteria, only tertiary treatment would significantly remove or kill bacteria. - Non-spore forming bacteria such as *E. coli* are found to survive some wastewater treatment plants. - Raw sewage overflow during flood conditions does occur in Australia. Data should be provided to support the conclusion that 'release of untreated sewage due to a leak or a storm overflow event is considered highly unlikely'. The RARMP may need to consider including additional risk management measures, e.g., ensuring participants are connected to tertiary wastewater treatment plants.	Discussion of the potential for the GMO to survive sewage treatment or to be released in untreated sewage was added to Step 1b of the likelihood assessment (Section 3.2 of Chapter 2 of the RARMP). Risk treatment measures have been imposed to reduce the likelihood of the GMO entering the environment via sewage (Section 2 of Chapter 3 of the RARMP).

Submission	Summary of issues raised	Comment
	• In step 2 of the likelihood assessment, the RARMP should discuss the uncertainty or provide evidence to support that GM bacteria are unlikely to establish if released. Propagule size and number of introductions may be more relevant for assessing vertebrate and invertebrate invasions not bacterial invasions. Peniston (2019) showed that propagule pressure might not be a good predictor of invasion success in prokaryotes. Additional data or evidence should be provided on the factors that will impact the potential establishment of the GMO on plant substrates.	Further information about the ability of the GMO to establish in the environment was added to step 2 of the likelihood assessment (Section 3.2 of Chapter 2 of the RARMP). Discussion of propagule size and propagule number has been removed.
	• In step 3 of the likelihood assessment, the RARMP should include further assessment of the ability of the GM bacteria to spread due to a selective advantage and compete with native non-GM bacteria in Australia. There is uncertainty on the growth or fitness and increased competition in the GM bacteria compared to the non-GM parent or native *L. brevis* strains. The risk of increased fitness should be assessed further and the uncertainty around the GM species addressed.	No further information was available regarding the ability of the GMO to compete with native non-GM bacteria in Australia. The likelihood of step 3 in the likelihood assessment (Section 3.2 of Chapter 2 of the RARMP) was revised to take high levels of uncertainty into account.
	• In step 4a of the likelihood assessment, the RARMP should clarify what levels of GM bacteria are expected on plant material and what levels may cause adverse effects on wildlife. There is uncertainty around what 'biologically relevant levels' are and what level would result in harm to animals.	Analysis of the potential exposure of animals to the GMO from consumption of fresh plant material, and whether these exposure levels could cause harm, was added to step 4a in the likelihood assessment (Section 3.2 of Chapter 2 of the RARMP).
	• In step 4b of the likelihood assessment, the potential for exposure of animals other than livestock should be considered. The risk of ruminant pest species e.g. deer, feral pigs, consuming fermented feed should be looked at as a possible pathway to spread.	The potential for wildlife to consume fermented feed was added to step 4b in the likelihood assessment (Section 3.2 of Chapter 2 of the RARMP). The potential for spread of the GMO via consumption and excretion by animals is considered in step 3 of the likelihood assessment.
	• Recommends that the cumulative likelihood assessment for risk scenario 3 is explained in more detail. Notes that the likelihood of steps and cumulative likelihood may be higher when the factors outlined above are considered.	The overall likelihood assessment (Section 3.2 of Chapter 2 of the RARMP) was rewritten to improve clarity. Revision of the steps of the likelihood assessment resulted in a higher overall likelihood estimate.

Submission	Summary of issues raised	Comment
	• The RARMP should include other potential adverse effects of VIP on animals or wildlife. The identified potential harms to people (not trial participants) or animals (livestock only) from VIP include immunosuppression or secretory diarrhea. VIP is present in many vertebrate species and exposure to VIP can cause other adverse effects. Data provided by the applicant indicate that the GM product may survive longer in the gut of small animals and therefore may have an increased impact compared to humans. Smalley et al (2009) states that VIP may have a detrimental effect of inducing allergy. The same author cites data that indicates that VIP may cause the increased survival, growth or reactivation of latent opportunistic bacteria. Avian prolactin (PRL) is under the control of VIP. Increased VIP and PRL are seen in mature males and is correlated with breeding behaviours such as nest defence and feeding young. PRL is a pluripotent hormone with many effects on growth, reproduction, migration, nurturing and feeding young. The possible impact of exposure to VIP on animal behaviour may need to be considered especially if there is any risk of widespread establishment in the environment.	VIP has many different effects in animals when secreted systemically or in specific organs. However, animals would only be exposed to the GMO via consumption. If consumed, both the GMO and its secreted VIP are expected to be localised in the gastrointestinal tract. The only known adverse effects of VIP in the gastrointestinal tract are immunosuppression or secretory diarrhea. There is no plausible way for the GMO or its secreted VIP to travel from the gastrointestinal tract to the brain and influence animal behaviour. As discussed in Section 4.1.1 of Chapter 1 of the RARMP, the synthetic VIP produced by the GMO is likely to have a longer half-life than natural VIP. This would have similar biological effects in the guts of humans and small animals. The Smalley et al paper reviews *in vitro* data indicating that VIP has a stimulatory effect on one type of innate immune cell, but inhibitory effects on the function of several other types of innate immune cells. Reviewed animal studies demonstrate that the overall effect of VIP is immunosuppression. The reported increased growth of opportunistic bacteria in the presence of VIP is an effect of immunosuppression. Lack of a fully functional immune system increases the likelihood of infections.
7	Accepts that, overall, Novotech's application has negligible risks to the health and safety of people and the environment. Satisfied that the measures taken to manage the short and long term risks from the proposal are adequate.	Submission has been noted.

Submission	Summary of issues raised	Comment
8	Agrees with the approach and considers that the risk posed by the GMO to the health of humans and the environment is minimal, providing that there is strict adherence to the proposed control measures, the requirements of the Therapeutic Goods Act 1989, compliance with the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and approval from the Department of Agriculture, Fisheries and Forestry for import of the GM treatment. Potential harms considered are appropriate to the use, the GMO and the controls proposed. The draft licence conditions proposed by OGTR will manage risks associated with importing, tracking and limiting the spread of the GMO and there are additional levels of control imposed by other regulators.	Submission has been noted.
9	• Too much VIP seems to cause secretory diarrhea, but according to the RARMP expression levels of VIP in the GMO have not been characterised. Surely, this would be characterised, otherwise, how would the applicant know the GMO is working?	The RARMP has been updated to clarify that expression levels of VIP have not been quantitatively characterised. There is data showing that the GMO produces VIP.
	• Does the expression cassette contain an antibiotic resistant gene for selective purposes?	This information is protected as confidential commercial information (CCI). It is included in the version of the RARMP that contains CCI.
	• Hope the applicant has a whole genome sequence of the parental strain.	The applicant has whole genome sequences of both the parental strain and the GMO. As discussed in Section 4.1 of Chapter 1 of the RARMP, presence of the intended genetic modifications and absence of any unintended insertions of exogenous sequence in the GMO were confirmed by whole genome sequencing.

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

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

Submission	Summary of issues raised	Comment
1	Concerned about dangers to human health from gene therapy, blood-brain crossing modified mRNA.	The proposed clinical trial does not involve gene therapy. The GMO is not able to enter human cells or alter the human genome. As discussed in section 4.1.1 of Chapter 1 of the RARMP, the GMO is modified to produce a synthetic human peptide. Many common medicines are based on human peptides, such as insulin for diabetics or oxytocin for inducing childbirth. The GMO is designed to treat inflammatory bowel disease. Therefore, the GMO will be orally administered to trial participants, and is expected to remain in the gastrointestinal tract rather than enter the bloodstream. There is no plausible way for the GMO or its mRNA to travel from the gastrointestinal tract to the brain.