



Australian Government

Department of Health

Office of the Gene Technology Regulator

June 2020

Risk Assessment and Risk Management Plan

for

DIR 171

Clinical trial of genetically modified Influenza
vaccine (H3N2 M2SR)

Applicant – Clinical Network Services (CNS) Pty Ltd

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 171

Decision

The Gene Technology Regulator (the Regulator) has received a licence application to conduct a clinical trial using a genetically modified organism (GMO). It qualifies as a DIR licence application under the *Gene Technology Act 2000* (the Act). The applicant, Clinical Network Services (CNS) Pty Ltd proposes to conduct a clinical trial to assess the efficacy and safety of GM influenza vaccines for protection of people from Influenza A virus infection.

Influenza (flu) viruses are highly infectious pathogens which are endemic in Australia. Influenza is predominately transmitted through aerosol droplets generated when an infected person coughs or sneezes, and influenza infections peak during the winter months. Symptoms usually present as a sudden onset of mild respiratory illness. In healthy individuals, infection normally resolves in under two weeks but the elderly, young children, pregnant women and the immunocompromised can suffer more severe symptoms.

The proposed GM vaccine is predicted to provide increased protection against influenza A virus infection. The GM vaccine would be manufactured overseas and imported into Australia. It would be administered by intranasal spray to a limited number of healthy children at clinical facilities located in Perth, Adelaide, Melbourne, Sydney or Brisbane.

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, CNS would require authorization from TGA before the trial commences. Clinical trials conducted in Australia must also be conducted in accordance with the [National Statement on Ethical Conduct in Human Research](#) and with the [Guidelines for Good Clinical Practice](#) of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

CNS would also require approval from the Department of Agriculture, Water and the Environment for import of the GM vaccine.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment, and that any risks posed by the dealings can be managed by imposing conditions on the trial.

The application

Project Title	Clinical trial of genetically modified Influenza vaccine (H3N2 M2SR) ¹
Parent organism	<i>Influenza A virus</i>
Principal purpose	To assess the safety and efficacy of GM influenza vaccine in a clinical trial
Genetic modifications	<p>Modified <i>Influenza A virus</i> gene conferring replication incompetence:</p> <ul style="list-style-type: none"> • <i>Insertion of two stop codons in M2 gene</i> – prevents virus assembly • <i>Deletion in M2 gene</i> – prevents virus assembly <p>Substituted <i>Influenza A virus</i> genome segments encoding antigens:</p> <ul style="list-style-type: none"> • <i>Hemagglutinin subtype 3</i> – influenza virus surface protein • <i>Neuraminidase subtype 2</i> – influenza virus surface protein
Previous clinical trials	<p>One completed phase 1 clinical trial in the United States</p> <p>Two ongoing phase 1 clinical trials in the United States</p> <p>One ongoing phase 2 clinical trial in Belgium</p>
Proposed limits and controls	
Proposed duration	3 years
Proposed trial size	Up to 240 clinical trial participants
Proposed locations	Up to four clinical facilities, which would be located in Adelaide, Brisbane, Melbourne, Perth or Sydney

Risk assessment

The risk assessment concludes that risks to the health and safety of people and the environment from the proposed clinical trial are negligible. No specific risk treatment measures are required to manage these negligible 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 included exposure of people or animals to the GMOs and whether there is the potential for reassortment with other viruses. Potential harms that were considered in relation to these pathways included ill health and increased disease in people or animals.

Important factors in reaching the conclusions of the risk assessment included: that the GM vaccine is replication incompetent; the inability of GMO progeny to be shed by the inoculated trial participants, and unintended exposure to the GMOs would be minimised by the limits and controls.

¹ The title of the project as supplied by the applicant is 'Clinical trials with a prophylactic influenza A/H3N2 live, M2-deleted, intranasal vaccine (H3N2 M2SR)'.

As risks to the health and safety of people, or the environment, from the proposed trial of the GM viruses have been assessed as negligible, the Regulator considers that the dealings involved do not pose a significant risk to either people or the environment.

Risk management plan

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

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a clinical trial, the licence includes limits on the size, location 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

Act	<i>Gene Technology Act 2000</i>
AEs	Adverse Events
AIVC	Australian Influenza Vaccine Committee
°C	Degrees Celsius
CDC	Centers for Disease Control and Prevention
cGCP	current Good Clinical Practice
cGMP	Current Good Manufacturing Practice
CNS	Clinical Network Services Pty Ltd
CRO	Clinical Research Organisation
Cth	Commonwealth of Australia
CTN	Clinical Trial Notification
CTX	Clinical Trial Exemption
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
EU	European Union
Eudra CT	European Union Drug Regulating Authorities Clinical Trials
dpi	days post inoculation
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
HA	haemagglutinin
HGT	horizontal gene transfer
HREC	Human Research Ethics Committee
IAV	Influenza A virus
IBV	Influenza B virus
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICH-GCP	<i>Guidelines for Good Clinical Practice</i> of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
M2	Matrix 2 protein
M2SR	M2 deficient Single Replication vaccine
NA	neuraminidase
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NHMRC	National Health and Medical Research Council
NLRD	Notifiable Low Risk Dealings
NPAAC	National Pathology Accreditation Advisory Council
OGTR	Office of the Gene Technology Regulator
ORF	Open Reading Frame

PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
PR8	Influenza A/Puerto Rico/8/34
QC	Quality Control
QLD	Queensland
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
SAEs	Serious Adverse Events
TCID ₅₀	Tissue Culture Infectious Dose 50%
TGA	Therapeutic Goods Administration
WHO	World Health Organisation

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The *Risk Analysis Framework* (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator ([OGTR website](#)).
5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed clinical trial are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

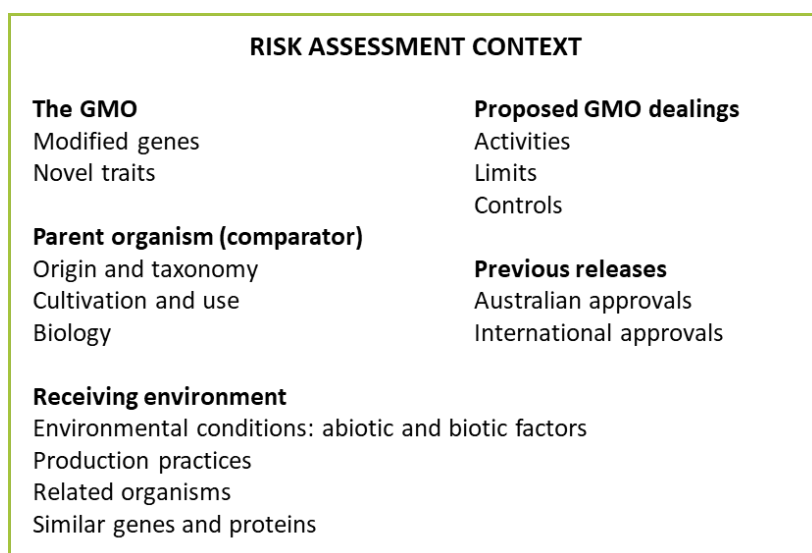


Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF

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.

1.1 Interface with other regulatory schemes

7. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Department of Agriculture, Water and the Environment (DAWE). Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.
8. 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 Exemption (CTX) scheme or the Clinical Trial Notification (CTN) scheme.
9. For clinical trials, the TGA has regulatory responsibility for the supply of unapproved therapeutic products. In terms of risk to individuals participating in a clinical trial, the TGA (as the primary regulatory agency), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) at each trial site all have roles in ensuring participants' safety under the *Therapeutic Goods Act 1989*. However, where the trial involves a GMO, authorisation is also required under gene technology legislation. To avoid duplication of regulatory oversight, and as risks to trial participants are addressed through the above mechanisms, the Regulator's focus is on assessing risks posed to people other than those participating in the clinical trial, and to the environment. This includes risks to people preparing and administering the GM virus, and risks associated with import, transport and disposal of the GMO.
10. The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects (ICH 1996). The guideline was developed with consideration of the current good clinical practices of the European Union (EU), Japan, and the United States of America (USA), as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). The TGA has adopted the ICH-GCP in principle as Note for Guidance on Good Clinical Practice (designated CPMP/ICH/135/95) (Therapeutic Goods Administration 2000), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.
11. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). This document sets the Australian standard against which all research involving humans is reviewed. The *Therapeutic Goods Act 1989* requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.
12. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical

trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and vaccine accounting and reconciliation.

13. The Department of Agriculture, Water and the Environment administers Australian biosecurity conditions for the importation of biological products under the *Quarantine Act 1908*. Biological products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines). Import of GM virus is subject to regulation by the Department of Agriculture, Water and the Environment and the Regulator.
14. All clinical trial sites would be located at medical facilities including out-patient settings, hospitals and associated pharmacies. Analysis of biological samples collected from trial participants administered with the GMO would occur at clinical trial sites, or at pathology laboratories. These facilities are regulated by State and Territory governments and adhere to professional standards for safety ([NSQHS](#)), disease control ([Australian Guidelines for the Prevention and Control of Infection in Healthcare \(2019\)](#)) and handling of pathology samples ([NPAAC](#)).

Section 2 The proposed dealings

15. Clinical Network Services Pty Ltd (CNS) has proposed clinical trials of a live GM replication incompetent Influenza (flu) vaccine. The purpose of the clinical trials is to assess the safety and efficacy of the GM vaccine in healthy volunteers. The GM vaccines would be manufactured overseas and imported into Australia. The GM vaccine would be administered to healthy children by intranasal spray, and samples that may contain GMOs would be collected from the trial participants for analysis in laboratories within Australia or exported for testing overseas.
16. The dealings involved in the proposed clinical trials are:
 - importing the GMOs;
 - conducting experiments with the GMOs;
 - transporting the GMOs;
 - disposing of the GMOs; and

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

2.1 The proposed limits of the trial (duration, scale, location, people)

17. The trial is proposed to take place over a three year period from the date of issue of the licence. The applicant intends to inoculate up to 240 participants with the GMO. Administration of the GM vaccine would not be carried out during the Australian influenza season (May to October).
18. The trial would take place at up to four clinical sites in Australia. While clinical sites have not been finalised, participating sites are likely to be located in Perth, Adelaide, Melbourne, Sydney and/or Brisbane.
19. Only trained and authorised staff would be permitted to conduct dealings with the GM vaccine.

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

20. The applicant has proposed a number of controls to minimise exposure to the GMO, and to restrict the spread and persistence of the GMOs in the environment. These include:

- ensuring the GM vaccine is administered by authorised, appropriately trained medical staff in clinical facilities in accordance with cGCP guidelines and standard precautions for working with potentially infectious material
 - requiring that clinical trial staff handling and/or administering the GM vaccine wear and use personal protective clothing and equipment
 - requiring that if any seepage of GMO from the nose occurs during the first five minutes after administration of the GMO, that it is to be collected with absorbent material and disposed of as infectious clinical waste
 - transport and storage of the GM investigational product and GM investigational product contaminated waste generated at a clinical trial site must be accordance with the current version of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
 - requiring decontamination of materials and equipment that have been in contact with the GMOs at clinical trial sites using effective disinfectants or disposal using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation
 - conducting the study outside of the Australian influenza season
21. Further information regarding the proposed controls is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

2.3 Details of the proposed activities

2.3.1 *Manufacture of the GMO*

22. The GMOs have been manufactured overseas in accordance with current Good Manufacturing Practice (cGMP).
23. The following relevant assays have been performed to ensure the quality and purity of the GMOs for the trial:
- Sequence analysis to confirm identity of the GM investigational product
 - Verification of absence of replication competent virus
24. Results of all tests met the pre-defined specifications and the applicant considers that the GM investigational product is suitable for use in clinical studies.

2.3.2 *Conduct of the clinical trial*

25. The international sponsor for the trial is FluGen Inc, which is based in the United States. CNS is applying for authorisation to conduct the proposed clinical trial in Australia and if the licence is approved, CNS would be responsible for ensuring that the licence conditions are met. The clinical trial would be managed through a clinical research organisation (CRO) within Australia.
26. The proposed clinical trial is a randomized, double-blind, placebo controlled Phase 1b study evaluating the safety and immunogenicity of the Sing2016 H3N2 M2SR vaccine in children. Immunogenicity would be assessed by measuring serum antibody responses by Hemagglutination-inhibition (HAI) and/or Microneutralization (MN) assays. Additional immune parameters would be assessed including mucosal antibody titres.

2.3.3 Selection of trial participants and behavioural requirements

27. In the proposed clinical trial, the vaccine would be administered intranasally to healthy children. In order to be enrolled in the trial, participants must meet the following relevant inclusion and exclusion criteria:
- trial participant and the parent(s)/guardian(s) must be willing to adhere to the requirements of the study and willing to communicate with the Investigator and understand the requirements of the study
 - trial participant must be judged as suitable by the Investigator, as determined by medical history, physical examination, vital signs and clinical safety laboratory examinations
 - trial participants must not have had a flu-like illness, or treatment for influenza in the previous 6 months
 - trial participants must not be confirmed or suspected to be immunosuppressed
 - trial participants must not have a significant history of seasonal hay fever, a seasonal allergic rhinitis, perennial allergic rhinitis, chronic nasal or sinus condition such as sinusitis, at the discretion of the investigator
 - trial participants must not have an acute febrile illness within 72 hours prior to vaccination
28. The applicant is not proposing to test trial participants for influenza infection, or the presence of other respiratory pathogens, as dosing would occur outside of influenza season in Australia. This strategy was used successfully in a previous clinical trial, conducted in the northern hemisphere. A total of 96 trial participants were tested for the presence of influenza RNA from nasal swabs taken prior to dosing, and none of the 96 participants tested positive for the presence of Influenza RNA.
29. The applicant is not proposing any special precautions to exclude subjects who have household or other close contacts with immunosuppressive conditions, as the GMO is replication incompetent and transmissibility of the GMO is expected to be very low (chapter 1 section 4.2.9).

2.3.4 Supply and storage of the GMO

30. The GMO would be imported according to International Air Transport Association (IATA) UN 3373 requirements for packaging and labelling.
31. Transport of the GM investigational product from customs in Australia would be directly to the clinical trial sites. Access to the area would be restricted to appropriately trained personnel. The secondary container would be labelled to indicate that it contains the GMO vials, the OGTR licence number and the contact details of an appropriate clinical trial staff member in case of loss of containment (who would on-report to CNS).
32. The proposed method of supply and storage of the GMOs, as advised by the applicant, would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

2.3.5 Intranasal administration of the GMO

33. Vaccine nasal sprayers would be transported in a sealed, unbreakable, leak-proof secondary container to the point-of-administration to the subject. The outer container would be labelled to indicate that it contains GMO, the OGTR licence number and contact details of an appropriate clinical trial staff member.

34. To administer the GM investigational product the nasal sprayer is inserted into the subject's nostrils and a fine spray is expelled into the nasal cavity. The nasal sprayer distributes the vaccine almost exclusively to the upper airway of the trial participant.
35. If there is seepage of the GMO from the nose of trial participants, then this would be collected with an appropriate absorbent material (e.g. tissue or similar) and disposed of in clinical waste as for other contaminated material (see chapter 1 section 2.3.9).
36. Parents and caregivers would be advised that in the event of a sneeze after administration a tissue should be used and properly disposed. Handwashing recommendations would be in place should there be a direct contact exposure. In the proposed study, vaccinated subjects would be instructed to remain at the clinic under observation for approximately 30 minutes post-administration. Site staff would remain within eye contact of vaccinated subjects for 15 minutes in case of any immediate reaction to the GM investigational product.
37. Participants would be administered the GMO in an outpatient setting in rooms in general treatment clinics that have containment equivalent to PC1 level.

2.3.6 Sample collection and analysis

38. Samples collected from trial participants may be analysed in Australia or overseas.

2.3.7 Personal protective clothing

39. The applicant advised that proper PPE, suitable for PC2 conditions, should be worn when working with the GMO. Clinical trial staff performing dealings with the GMO including administration of the GMO to trial participants and clean-up of potential spills would wear a gown, gloves and eye protection (safety glasses or face-shields).

2.3.8 Transport of the GMO

40. Samples may be transported to a third-party testing laboratory within Australia or transported for export to overseas laboratories for analysis. As no infectious virus has been detected in nasal swabs collected on days 1, 2, 3 and 7 post-dose in any subject vaccinated with a similar live GM Influenza vaccine (chapter 1 section 4.2.8), the applicant is proposing that the samples would be handled using standard packaging and labelling of infectious laboratory samples. This is discussed in chapter 2, section 2.4.1.
41. Waste generated at clinical trial sites would be transported from clinical waste bins by waste contractors for incineration.

2.3.9 Decontamination and disposal of the GMOs (including waste contaminated with the GMOs)

42. All unused GM investigational product and disposable materials used during the administration procedure or for the collection of samples from participants and any other contaminated waste (e.g. sprayers, gloves, needles, tissues) would be disposed of according to infectious medical waste management procedures. The outer container of GMO waste would be labelled to indicate that it contains GMOs. Commercial waste management contractors would be used. All disposable GMO waste would be destroyed by high-temperature incineration.
43. Waste contractors would be selected based on their experience and capability in disposing of infectious clinical waste and laundering/disposing of linen which has been contaminated with

infectious substances. Waste contractors would handle GMO contaminated waste using the same safety precautions for handling infectious waste.

44. Contaminated work clothes are to be laundered before re-use and are not to be taken home.
45. Spills of the GMO would be decontaminated using a fresh dilution of 1% to 10% sodium hypochlorite (bleach), with a minimum contact time of 30 minutes.

2.3.10 Training of clinical trial personnel

46. CNS would have responsibility for ensuring training of personnel and compliance with OGTR licence conditions.
47. All clinical trial staff would be trained in Good Clinical Practice (GCP) requirements.
48. Persons handling the GMO during administration (i.e. the Principal Investigator, the Study Coordinator and medical staff assisting in administration of the GMO to participants), would be trained in licence conditions and all procedures specific to the GMO including handling, spill procedures, containment and disposal. Records of this training would be kept within the clinical trial master file. A copy of the licence would also be kept in the clinical trial file at the site.
49. The appropriate clinical trial staff member whose contact details are listed on the outer container(s) of GMO investigational product would also be trained in the conditions of the licence including the requirement to report loss of containment to the OGTR and the procedure for doing so.
50. Couriers would be informed they are transporting a GMO via the labelling on the outer container. In addition, a copy of the licence would be included in the shipping documentation.

2.3.11 Contingency plans

51. In case of unintentional release of the GMO due to an accidental spill, the spill would be reported to CNS by clinical trial staff trained in the OGTR reporting requirements. CNS would on-report to the OGTR. The local IBC would also be notified of loss of containment or suspected loss of containment.
52. Spill clean-up procedure: Allow aerosols to settle, gently cover spill with absorbent material and apply 1% sodium hypochlorite and allow 30 minutes contact time with the disinfectant before clean up.
53. In case of exposure of people to the GMO: wash off immediately with water and soap, remove all contaminated clothing. In case of eye contact, rinse with water for at least 15 minutes

2.3.12 Accountability and Monitoring

54. Every primary container of the GM investigational product would be accounted for, in line with standard clinical practice.
55. Severe adverse Events (SAEs) occurring at any time during the study would be recorded. Reported Adverse Events (AEs) would also be recorded for each participant following vaccination with the GM investigational product. A Safety Monitoring Committee would be available to review available safety data at specified time points and as needed.

56. Further information regarding the conduct of the trial is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

Section 3 Parent organism – Influenza virus

57. The parent organism is the human Influenza A virus. The characteristics of the non-GM parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of influenza virus will be discussed here.
58. Human influenza A and B viruses are highly infectious viruses that cause human influenza (flu), a contagious disease of the respiratory system. Flu viruses generally transmit through large aerosol droplets that are generated when an infected person coughs, sneezes or talks. They are also transmitted when contaminated surfaces, such as hands or tissues, make contact with the mucous membranes.
59. In temperate climates, the annual influenza epidemic peaks during winter while in the tropics, it can occur throughout the year. The annual attack rate or proportion of people who become ill after exposure is estimated at 5%–10% in adults and 20%–30% in children (WHO, 2018). Influenza viruses are endemic in Australia (Health, 2015).
60. The onset of flu is sudden and it is accompanied by malaise, persistent runny nose, cough, headache, sore throat and high fever. Infection normally resolves in less than two weeks without the need for treatment in healthy individuals. Symptoms may be reduced if antiviral drugs are administered within 48 hours of initial symptoms (Stiver, 2003). Fatalities can occur when individuals who are weakened by influenza develop pneumonia and bronchitis from a secondary bacterial or viral infection.
61. Those at highest risk of the more severe symptoms include the elderly, young children, pregnant women and the immunocompromised. Influenza generally aggravates respiratory conditions such as asthma.
62. Shedding of detectable amounts of influenza virus from the respiratory tract generally begins one day after infection, with symptoms appearing two days after infection. Viral replication peaks approximately two days after infection and declines slowly from there. Shedding typically continues for a further three to five days and can last up to nine days in healthy adults. Shedding does not significantly differ between influenza types or subtypes. Shedding in young children lasts for a longer time period and generally occurs up to seven days (WHO Writing Group, 2006; Carrat et al., 2008; Suess et al., 2010).
63. Recovery of viable influenza viruses from stool samples has rarely been reported (Minodier et al., 2015; Minodier et al., 2019), indicating that the respiratory tract may be the primary source of shedding, as the levels of viral RNA detected from nasal swabs was much higher than the levels detected in stools (Minodier et al., 2019).
64. Influenza viruses are rarely detected in blood using PCR methods. However, when influenza is detected by PCR, it is generally in severely ill patients, who required hospitalisation (Tse et al., 2011; Stramer et al., 2012; Suess et al., 2012).
65. The biology of Influenza A and B viruses has been described in detail in the RARMPs for DIR-137 and DIR-144 (clinical trials with GM Influenza viruses).

3.1 Genome and virion structure of influenza A virus

66. The genome of influenza A viruses is made up of eight single-stranded, negative-sense RNA segments as shown in Table 1.

Table 1. Segments of influenza A virus

Segment number	Segment code	Largest protein encoded by segment
1	PB2	polymerase basic 2
2	PB1	polymerase basic 1
3	PA	polymerase acidic
4	HA	haemagglutinin
5	NP	nucleoprotein
6	NA	neuraminidase
7	M	matrix
8	NS1	non-structural protein

67. The RNA segments do not exist as naked RNA but are always associated with multiple copies of viral nucleoprotein (NP) that protect it from host ribonucleases. Each genomic segment exists as a ribonucleoprotein (RNP) with the viral RNA wrapped around the outside of the nucleoprotein (NP) oligomer and attached to the polymerase complex (Figure 2).

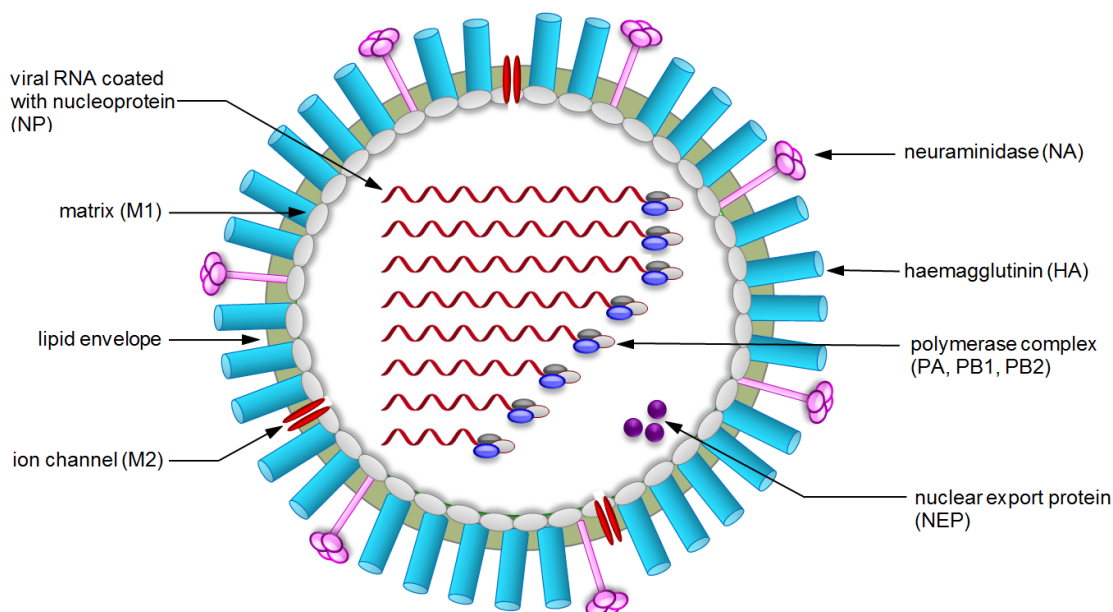


Figure 2. Schematic representation of the influenza virus particle

68. The eight RNPs are enclosed in a layer of matrix protein (M1). M1 is the most abundant protein in the virus; it drives virus budding and controls the intracellular trafficking of RNPs. The viral envelope, which is a lipid bilayer derived from the host cell membrane with viral proteins inserted, lies just outside the M1 layer.

69. The lipid envelope has three integral membrane proteins, namely haemagglutinin, neuraminidase and proton-selective ion channel (M2). Haemagglutinin and neuraminidase are the major antigenic determinants on the surface of the influenza virus. The M2 proton channels are essential for uncoating and budding of viral particles.

3.2 Haemagglutinin and its role in cellular entry of influenza viruses

70. Haemagglutinin has two main functions: receptor binding and facilitating fusion of viral and host membranes. This transmembrane protein is the most abundant protein on the virion surface. Human influenza A viruses have one of three types of haemagglutinin, H1, H2, or H3.
71. The major target cells for human influenza viruses are epithelial cells lining the respiratory tract. The exposed surfaces of these cells are glycosylated and the glycans have sialic acid which is the receptor for influenza viruses.
72. Newly budded viruses are not infectious as their intact haemagglutinin (HA0) must first be activated by proteolytic cleavage into two peptides (HA1 and HA2). Cleavage generates a short hydrophobic sequence at the N-terminus of HA2 called the fusion peptide and this peptide is required to initiate fusion of viral and host membranes. HA1 forms the receptor binding site for the host sialic acid receptors. HA1 and HA2 remain intertwined after cleavage. Since receptor binding and infection cannot proceed in the absence of the fusion peptide, the pathogenicity of any viral subtype is determined, in part, by the ease of HA0 cleavage.
73. After receptor binding, influenza viruses enter the host cell either by receptor mediated endocytosis or macropinocytosis, depending on their morphology (Rossman et al., 2012). These entry mechanisms are triggered when a virus attaches to the cell surface. The internalised virus is encapsulated in an endosome and at this point, the viral RNPs are separated from the host cytoplasm by the endosomal membrane, the viral envelope and the capsid.
74. Release into the cytoplasm requires the fusion of the viral and host (endosomal) membranes but membrane fusion is an energetically unfavourable process. Mediation of membrane fusion is the second function of haemagglutinin. The host cell acidifies the contents of the endosome to enable destruction by acid hydrolases. The drop in pH triggers a conformational change in haemagglutinin. This exposes the previously buried fusion peptide, which then inserts into the endosomal membrane, resulting in haemagglutinin being attached to both membranes. Several haemagglutinin trimers, acting in concert, distort the membranes and pores form, allowing membrane fusion of the host endosome membrane and the viral envelope.

3.3 M2 proton-selective ion channel is required for viral uncoating and budding

75. The virus must be uncoated concurrently with membrane fusion. When the pH drops in the endosome, this activates the pH sensitive M2 proton-selective ion channel. (reviewed in Manzoor et al., 2017). When activated, the M2 ion channel allows protons to enter the interior of the virus particle. Acidification of the virus particle interior disrupts protein-protein interactions resulting in disassociation of M1 proteins from the virus envelope and viral RNPs.
76. The net result of the two events triggered by endosome acidification (conformational change of HA and activation of the M2 ion channel) is the release of the viral RNPs into the cytoplasm of the infected cell. The subsequent import of the viral RNPs into the nucleus allows viral replication to begin.
77. The M2 ion channel also has roles in virus assembly and budding. During budding, M2 ion channels localize at the 'neck' of the bud, between the newly forming virion and the plasma

membrane of the host (Rossman et al., 2010a; Rossman et al., 2010b). The M2 ion channels at the neck of the bud are thought to cause positive curvature of the membrane, resulting in membrane scission and release of the new virion particle from the host plasma membrane (Rossman et al., 2010b).

78. In the absence of functional M2, budding virions still form, however membrane scission and virion release does not occur. This results in the formation of long, filamentous buds, or “beads on a string” phenotypes observed in viruses lacking functional M2 (Iwatsuki-Horimoto et al., 2006; Rossman et al., 2010a; Roberts et al., 2013).

3.4 Viral replication

79. Viral replication does not involve a DNA intermediate. The negative sense genomic RNA (vRNA) serves as the template for the synthesis of both mRNA and the complementary genomic strand of RNA (cRNA). cRNA is the full length transcript of vRNA while mRNA is a truncated transcript. Transcription and replication of the influenza virus occur in the nucleus.
80. Influenza viruses replicate very quickly. Host cells start shedding new progeny viruses from around 6 hours after infection (WHO, 2015).
81. Up to 90% of virus-infected cells fail to release infectious progeny. Analysis of viral progeny shows that propagation-competent virions containing one each of the eight RNPs are outnumbered by semi-infectious virions with an incomplete set of RNPs (Brooke et al., 2014). The propagation-competent fraction of virions varies widely between different strains of influenza virus.

3.5 Neuraminidase

82. Neuraminidase hydrolyses the glycosidic bond between sialic acid (N-acetyl neuraminic acid) and galactose.
83. Haemagglutinin and neuraminidase are glycoproteins, and sialic acid is present as a terminal sugar in their glycans. Without neuraminidase, large aggregates of viruses form at the surface of the infected cell, due to binding between haemagglutinin on the newly budded viral particles and sialic acid on the cell surface, as well as between haemagglutinin and sialic acid on adjacent particles. Aggregation of viral particles is the main reason flu viruses do not spread as quickly in the absence of neuraminidase (Palese et al., 1974; Liu et al., 1995).
84. The respiratory epithelium, which is the target tissue of influenza viruses, is protected by a layer of mucus up to 50 µm thick. The main protein in mucus is mucin, a highly glycosylated protein with sialic acids decoys that mimic the true receptors on epithelial cells. Influenza viruses that bind these ‘decoy’ receptors are trapped in the mucus and removed during mucus clearing, which is part of the innate defence system. Neuraminidase frees the viruses from binding of the decoy receptor, thereby enabling them to penetrate the protective mucus layer during infection.
85. Neuraminidase also increases virulence of flu viruses by compromising the immune defences at the mucosal surface. It removes sialic acid from T-cells in the mucosa and from immunoglobulin A (IgA)-producing B cells, adversely affecting their function. It also de-sialidates IgA, resulting in its being cleared more quickly by the hepatic system (Bhatia and Kast, 2007).
86. Two neuraminidase subtypes are found in human influenza A viruses.

3.6 Mutation and reassortment

87. Point mutations in the main antigenic determinants, haemagglutinin and neuraminidase, result in antigenically-novel viruses that can cause disease in previously resistant or immune hosts. The creation of novel antigenic combinations is known as antigenic shift.
88. Point mutation rates are very high in single stranded RNA viruses, estimated at two to three errors per replicated genome (Drake, 1993; Sanjuan et al., 2010; Pauly et al., 2017).
89. When a cell is co-infected, or 'Superinfected' with two influenza viruses of the same type (i.e. two influenza A viruses or two influenza B viruses), each of the eight vRNPs in the progeny virus can originate from either infecting virus because the genome is segmented. Such viral progeny are called reassortants.
90. There are many factors that influence the frequency of novel reassortant viruses. Reassortment is dependent on the occurrence of co-infection of Influenza A virus (IAV)s. The frequency of co-infection is dependent upon the viral dose received, with higher doses resulting in increased co-infection rates *in vitro* and *in vivo* (Bodewes et al., 2012; Marshall et al., 2013). In addition, viral spread of the first infection prior to inoculation with the second infection can increase the probability of co-infection and reassortment. Both co-infection and reassortment rates were reduced in cell culture when the first infection occurred in conditions that did not allow for viral spread (Marshall et al., 2013).
91. Another factor affecting the rate of co-infection is the time delay between the primary and secondary infections. Co-infection is strongly inhibited in a time-dependent manner, with inhibition starting to take effect by 2, 6 or 12 hours post infection *in vitro* (Huang et al., 2008; Marshall et al., 2013; Dou et al., 2017; Sun and Brooke, 2018). The range in inhibition start times found in the literature may be due to experimental differences, such as doses used. When guinea pigs were intranasally inoculated with two IAVs with a time delay of 12 hours between infections, nearly half of the virus isolates were found to be reassortants. However, when the time delay was increased to 24 hours between infections, none of the virus isolates were reassortants (Marshall et al., 2013). These data suggest that there is a small temporal window where co-infection with two different IAVs can occur.
92. Multiple factors can promote or prevent the establishment and spread of reassortant viruses. Generally, more frequent reassortment is thought to take place between IAV strains that are more genetically similar, and the more divergent they are, the less likely reassortants are to establish and spread (Marshall et al., 2013; Brooke, 2017; Phipps et al., 2017; Villa and Lassig, 2017). There may also be genetic incompatibilities between parental strains that result in attenuated progeny, with reduced fitness, such as incompatibilities between the three polymerase segments (Phipps et al., 2017) or an HA/NA imbalance (chapter 1 section 3.8). Consequently, differences between parental strains can limit reassortment or heavily bias the production of reassortants with specific gene segment combinations.
93. Studies on the occurrence of reassortants in humans have suggested that there is both negative selection against reassortants, and restriction in the gene segment combinations being produced, reducing the effective rate of reassortment to much lower levels than has been reported in animals (Sobel Leonard et al., 2017; Villa and Lassig, 2017). This was suggested to be in part due to lower doses of IAVs received by the humans compared to animals and the fact that humans have a much larger respiratory tract surface area than guinea pigs or ferrets, which may result in lower co-infection rates (Sobel Leonard et al., 2017).

94. A cell could be co-infected with influenza A viruses from two different host organisms e.g. a human influenza virus and a swine influenza virus (chapter 1 section 3.7). These two influenza A viruses could reassort, resulting in a novel combination of the antigenic determinants HA and NA. While the majority of possible reassortants would be expected to be non-viable and have reduced fitness compared to the parental strains, some novel combinations could potentially lead to the emergence of new influenza strains (chapter 1 section 3.7).

3.7 Host range

95. Influenza viruses are generally host specific. The principal reservoir of human influenza A viruses is humans but new human subtypes can arise from avian reservoirs.
96. Direct bird to human transmission is not common and it has not resulted in a sustainable pathogen as avian subtypes transmit poorly between humans. To cross the species barrier, the avian virus must acquire changes in the receptor specificity of haemagglutinin and neuraminidase, and replicate efficiently at the lower human body temperature. Avian influenza virus replication is somewhat restricted by the 32°C ambient temperature of the human nose. The temperature of the avian gut, where the receptors are present and replication would occur, is estimated at 41°C.
97. Human infections with avian influenza viruses generally occur via domesticated intermediates. These infections have not resulted in sustained human to human transmission. The greatest risk occurs during the handling and slaughtering of live infected poultry. Proposed routes of infection include the inhalation of infectious aerosols or aerosolised faeces, and contact with contaminated surfaces.
98. Companion animals such as dogs and cats have sialic acid receptors that are similar to those of birds and they are able to be infected with avian influenza. Dogs and cats are unlikely to be infected with human influenza viruses, as human influenza viruses are adapted to infect cells with human-like sialic acid receptors. In rare isolated cases, dogs and cats have been infected with human influenza viruses (Borland et al., 2020). A human-like H3N2 influenza virus has been isolated from dogs. These dogs shed the virus, had fever, sneezed and coughed (Chen et al., 2015). Cats can be infected with H3N2 canine influenza (Song et al., 2015). Infected cats shed the virus, had elevated temperatures and severe pulmonary lesions.
99. Outbreaks of influenza occur sporadically among farmed animals including swine and mink (Gagnon et al., 2009). Swine have both avian-like and human-like sialic acid receptors and consequently, influenza viruses are able to be transmitted from swine to humans, if there is close contact (e.g. pig farmers). Subsequent person-to-person transmission is very limited (Olsen et al., 2002). The viruses may also transmit from humans to swine (Shin et al., 2006).
100. In Australia, the outbreak of equine influenza in 2007 and of avian influenza (H7N2) in 2013 did not result in human infections. Horses have a sialic acid receptor that is similar to that of avian species (Suzuki et al., 2000).

3.8 The haemagglutinin-neuraminidase balance

101. The levels of haemagglutinin and neuraminidase activity have to be very finely balanced for productive viral infection. Haemagglutinin and neuraminidase both bind sialic acid but have opposing functions: haemagglutinin binds the receptor for cellular entry while neuraminidase cleaves the receptor to free the virus. If the level of neuraminidase activity is too high, the receptor will be cleaved before the virus can undergo endocytosis and the host cell will not be infected. Conversely, if the level of neuraminidase activity is too low, the receptor will be cleaved

too slowly during budding, viral progeny will aggregate and be prevented from infecting other cells. Some haemagglutinin-neuraminidase combinations recur while others are rarely observed in both natural and laboratory-derived reassortants (see chapter 1 Section 3.6 for discussion of reassortment). The replication fitness of these reassortants could be explained by a mismatch in receptor binding and release (Wagner et al., 2002).

3.9 Environmental stability and decontamination methods for influenza virus

102. Influenza viruses remain viable on non-porous surfaces such as stainless steel and plastic for up to 24 hours, and on semi porous surfaces such as cloth, paper and tissues for eight to twelve hours (Bean et al., 1982).

103. Influenza viruses can be effectively inactivated using many commonly used disinfectants and heat treatment. Washing hands with soap and water can prevent contact transmission of the virus, as this disrupts the viral lipid envelope. Surfaces can be chemically decontaminated with standard disinfectants such as bleach, 70% ethanol, 2% alkaline glutaraldehyde and 5 to 8% formalin. Physical decontamination includes moist heat at 121°C for 20 minutes or dry heat at 70°C for 5 minutes, 80°C for 2.5 minutes or 90°C for 1 minute (Jeong et al., 2010; Pathogen Regulation Directorate, 2011a, b).

3.10 Antiviral treatments for influenza virus

104. Antiviral agents are 70-90% effective as short term prophylactics (Monto, 2003). Neuraminidase inhibitors such as oseltamivir (Tamiflu) and zanamivir (Relenza) may shorten the period of influenza infection.

105. M2 inhibitors such as amantadine and rimantadine, block the M2 ion channel and in doing so, prevent uncoating of the virus and progression of the infection. The spread of a single mutation in the M2 protein has resulted in widespread resistance to this class of drugs.

3.11 Flu vaccines

106. Annual vaccination against circulating flu strains is strongly recommended for high risk groups such as the elderly and the immunocompromised (Centers for Disease Control and Prevention, 2008). The *Australian Immunisation Handbook* recommends annual influenza vaccination for everyone 6 months of age and over (Australian Technical Advisory Group on Immunisation (ATAGI), 2018).

107. The most common flu vaccines are inactivated (killed) vaccines, which can be divided into whole virus vaccines, split virus vaccines and subunit vaccines. In whole virus vaccines, an immune response is elicited by intramuscular injection of the intact but killed virus. Split virus vaccines use whole virus that has been disrupted by a detergent. By comparison, subunit vaccines only use partially purified haemagglutinin and neuraminidase protein. Inactivated vaccines do not offer effective protection against influenza viruses that have shifted antigenically from the recommended target strains or promote cellular immunity. Unlike inactivated vaccines, live attenuated flu vaccines are able to provide broad cross-protection against antigenically divergent influenza strains.

108. The influenza vaccine virus strain is updated every year to provide coverage and protection for the circulating virus strains. Due to the combination of high mutation rates and antigenic selection driving mutations in haemagglutinin and neuraminidase, circulating virus strains are always changing. Consequently, the WHO recommends flu strains for targeting with vaccines (targeted strains) twice annually. Recommendations are made in February for the Northern

Hemisphere flu season and in September for the Southern Hemisphere flu season. The WHO's recommendations are evaluated by the Australian Influenza Vaccine Committee (AIVC) which provides advice to the TGA on the composition of the seasonal flu vaccine to be supplied each year in Australia.

3.12 Risk group of influenza virus type A

109. The Australian Standard 2243.3:2010 *Safety in Laboratories Part 3: Microbiological safety and containment* (Standards Australia/New Zealand, 2010) classifies influenza as a Risk Group 2 organism. Highly pathogenic strains of Influenza are classified as Risk group 3 organisms.

Section 4 The GMO – nature and effect of genetic modifications

110. This application proposes a trial for a live GM flu vaccine using an influenza A virus strain referred to as "Sing2016 M2SR" that has been genetically modified to improve its safety while still eliciting an immune response.

4.1 The genetic modifications

4.1.1 *The Influenza A/Puerto Rico/8/34 (PR8) parental strain*

111. The parent organism is the Influenza A/Puerto Rico/8/34 (PR8) strain of human influenza A virus. This strain has been used as a donor backbone virus for decades in traditional inactivated influenza vaccine manufacturing, and has a favourable safety profile. PR8 has undergone extensive passaging in eggs, mice and ferrets, resulting in a virus that is severely attenuated for humans and is unlikely to cause harm to human health (Beare et al., 1975). As mentioned previously, the Influenza A virus genome is composed of 8 segments of RNA. Influenza vaccines manufactured using the PR8 strain as a donor backbone use six of the RNA segments from PR8 and replace the two RNA segments encoding the HA and NA antigens with the two corresponding RNA segments from recommended seasonal target strains for vaccination. The new 6:2 reassortant virus can then be used to manufacture inactivated flu vaccines to provide protection from the recommended target strains.

4.1.2 *M2SR vectors for production of live replication incompetent flu vaccines based on PR8*

112. To create a live, replication incompetent vaccine using the PR8 strain, the gene encoding the M2 ion channel, which is essential for replication of the influenza virus was genetically modified to be non-functional. This was achieved by inserting two stop codons into the transmembrane domain of the M2 open reading frame. This modified virus is referred to as M2 Knock Out (M2KO) and is described in Watanabe et al. (2009). The M2KO virus is highly attenuated and only very low levels of replication were able to be detected in mammalian cells.
113. The M2 deleted Single Replication (M2SR) vector is based upon the original M2KO virus. The M2SR vector differs from M2KO as it has a 51 nucleotide deletion in the transmembrane domain of M2, following the two stop codons described in the original construct (Sarawar et al., 2016). Therefore, even if a read through of the two stop codons occurs, no functional, full-length M2 protein would be made (see Figure 3). M2SR is replication incompetent in normal mammalian cells, but is able to replicate in mammalian cells that complement the GMO by stably expressing M2 protein.

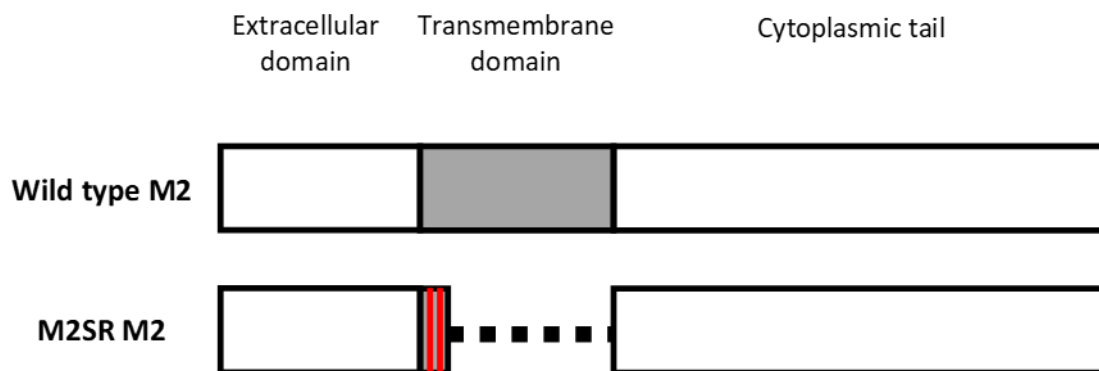


Figure 3. Schematic of wild type M2 gene and the genetic modifications made to the M2SR M2 gene.

114. The extracellular domain and the cytoplasmic tail of M2 are shown in white, while the transmembrane domain is shown in grey. Two red lines represent the two stop codons inserted at the beginning of the transmembrane domain. Dotted line indicates the 51 nucleotide deletion in the transmembrane domain of M2.

4.1.3 Generation of high-yield M2SR vectors

115. The M2SR vector to be used in this study has been further improved to increase virus yield for manufacturing purposes. M2SR virus was generated with changes that allow for increased growth in cell culture. Some of the multiple changes generated in the M2SR virus have been previously described as contributing to influenza virus growth in cell culture in the PR8 vaccine virus background (Ping et al., 2015). Although the relative contributions of each change to the high-yield phenotype has not been determined, it is the combinatorial effect of these changes which is thought to result in the high yield phenotype.

4.1.4 Insertion of HA and NA from A/Singapore/INFIMH-16-0019/2016 (H3N2)

116. The GM flu vaccine to be used in the proposed clinical trial is a 6:2 reassortant virus using the 6 RNA segments from the high-yield M2SR vector and the 2 RNA segments encoding HA and NA from the A/Singapore/INFIMH-16-0019/2016 reference virus, the H3N2 strain recommended by WHO for inclusion in the influenza vaccines for the 2018-2019 northern hemisphere influenza season and is referred to here as “Sing2016 M2SR”.

4.2 Characterisation of the GMO

117. Data obtained from experiments using the proposed GMO Sing2016 M2SR, and from other vaccine viruses generated using similar backbones (high-yield M2SR, M2SR or PR8 backbones) with alternative HA and NA segments derived from different strains of Influenza A viruses has been taken into consideration to assess the characteristics of the GMO.

118. The prototype monovalent vaccine initially used for testing in humans was generated using the original M2SR virus backbone with HA and NA segments derived from an A/Brisbane/10/2007-like H3N2 virus, which was recommended by WHO for inclusion in the influenza vaccines for the southern hemisphere for the 2008 and 2009 influenza seasons. This GM vaccine is referred to

here as “Bris10 M2SR”. Approved human clinical trials using Bris10 M2SR conducted overseas are listed in Table 2 below.

4.2.1 Viral replication of the GMO *in vitro* and *in vivo*

119. The high-yield Sing2016 M2SR vaccine was found to be unable to replicate in normal mammalian cells, and must be grown in cells that stably express M2 protein in order for replication to occur. The GMO would be expected to be replication incompetent, irrespective of the immune status of the host.
120. Further information regarding replication of the GMO *in vivo* is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

4.2.2 Virulence of the GMO *in vivo*

121. Ping et al. (2015) compared the virulence of replication competent PR8 virus and a replication competent high-yield PR8 virus, which shares some of the changes in common with the high-yield M2SR. The intranasally administered doses required to kill 50% of the infected mice were similar for the PR8 virus and the high-yield PR8 virus ($10^{2.5}$, 10^2 , respectively). However, as compared to mice infected with the PR8 virus, mice infected with the high-yield PR8 virus generally lost slightly more weight and had higher virus titres in the lungs on day 5 post infection than did mice infected with the PR8 virus. These observations suggest that the increased replicative ability of the high-yield PR8 viruses may cause them to be slightly more virulent than the original PR8 strain *in vivo* (Ping et al., 2015). Unlike the change in viral titres, the decrease in weight was not considered statistically significant due to the small sample size. This correlation would have to be verified with a larger sample size.

4.2.3 Genetic stability of the GMO

Serial passaging and production lots

122. The applicant reported that high-yield M2SR viruses were serially passaged in cells permissive for the vaccine virus for 20 passages. Sequence analysis at passage 20 showed a single mutation in one of the six segments from PR8, indicating that the backbone is genetically stable.
123. M2SR viruses without the high-yield changes (chapter 1 section 4.1.2) were demonstrated to be genetically stable and maintained replication incompetence (Sarawar et al., 2016). Sequencing of the M RNA segment after 20 passages of M2SR viruses in cells expressing the M2 protein revealed that the virus still possessed the two introduced stop codons and the deletion in the transmembrane domain. 20 blind passages of M2SR in non-permissive, normal cells, not expressing the M2 protein to select for potential escape mutants did not generate any revertant viruses.

Reassortment studies

124. When mice were intranasally administered with both M2SR virus vaccines and live wild type Influenza viruses at the same time, no replication-competent viruses containing M2SR segments were observed (Y.Hatta & P.Bilsel, unpublished data, referred to in Hatta et al. (2017)). This indicates that the GMO is unlikely to reassort and gain replication competence *in vivo* when co-infection with a second replication competent virus occurs. This may be due, in part, to the non-spreading, replication incompetent property of M2SR viruses.

125. Further information regarding the genetic stability of the GMO is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

4.2.4 Nonclinical studies

126. Studies in mice and ferrets using M2SR based vaccines have demonstrated that these live, replication incompetent vaccines provide effective protection against both homologous (same subtype, based on the classification of haemagglutinin (H) and neuraminidase (N) used in the vaccine) and heterologous (different subtype to vaccine) strains of influenza A viruses (Sarawar et al., 2016; Hatta et al., 2017).
127. The ability of M2SR based vaccines to provide protection against heterologous influenza A strains is thought to be due to production of cross-reactive antibodies against the conserved HA2 stalk region, which was found to occur in both mice and ferrets (Sarawar et al., 2016; Hatta et al., 2017). This suggests that M2SR based vaccines could offer a major advantage over currently approved vaccines, which offer little or no protection against newly emerging or mutated circulating influenza A strains.
128. In addition, it was observed in ferrets that while a single dose of the vaccine provided protection against challenge with influenza virus, a two dose regimen using a priming dose and a booster dose 28 days later provided better protection (Hatta et al., 2017).
129. M2SR based vaccines were able to provide effective protection against heterologous influenza A strains in both naïve ferrets and also in those with pre-existing immunity (Hatta et al., 2018).

4.2.5 Clinical studies

130. No clinical studies with the proposed GMO have been performed in Australia, however, four clinical studies using M2SR based vaccines have been approved overseas (chapter 1 section 6.2, Table 2). One of the approved ongoing trials FLUGEN-H3N2-V003, involves high-yield Sing2016 M2SR, the GMO proposed for use in this licence application. All four of the approved human clinical trials have involved the use of the prototype original M2SR vaccine Bris10 M2SR. The active phases for first two studies have been completed (FLUGEN-H3N2-V001 and FLUGEN-H3N2-V002) (Eiden et al., 2018; Eiden et al., 2019), while the remaining two studies are still underway.

4.2.6 Immunogenicity

131. In the Phase I dose-ranging study, FLUGEN-H3N2-V001, adult trial participants were intranasally inoculated with Bris10 M2SR at dose levels of 10^6 , 10^7 or 10^8 TCID₅₀. A dose-response effect for the production of humoral (HA antibody) and mucosal antibodies was observed (Eiden et al., 2018).
132. In addition, in the Phase IIa challenge study, FLUGEN-H3N2-V002, 48 adult trial participants who received 10^8 TCID₅₀ of the Bris10 M2SR vaccine were subsequently challenged 4 weeks later with 10^6 TCID₅₀ of an antigenically distinct wild type influenza virus, A/Belgium/4217/2015 (H3N2). Inoculation of trial participants with Bris10 M2SR was found to protect against influenza infection from the antigenically distinct challenge strain, indicating the potential for improved breadth of protection by M2SR in humans, similar to results obtained in ferrets (chapter 1 section 4.2.4). Inoculated trial participants had reduced viral loads and reduced illness symptoms after challenge with the wild type influenza virus compared to trial participants who received the placebo (Eiden et al., 2019).

4.2.7 Adverse events

133. Across the Phase I dose-ranging study and the Phase IIa challenge study, a total of 72 adult trial participants have received the highest dose of Bris10 M2SR tested (10^8 TCID₅₀) with no serious adverse events (SAEs) or adverse events (AEs) of significance that would halt these studies (Eiden et al., 2018; Eiden et al., 2019).
134. Trial participants inoculated with the Bris10 M2SR vaccine have shown mild AEs, most commonly runny noses and/or nasal congestion during the first 7 days after vaccination. No fevers ($\geq 38^\circ\text{C}$) were reported in any trial participants. In trial participants administered a dose of 10^8 TCID₅₀ of Bris10 M2SR, 83% of trial participants reported at least one AE, however the frequency of reported AEs in the placebo group was also relatively high, at 46% (Eiden et al., 2018).
135. The two ongoing studies are FluGen-H3N2-V003 and NIAD (see Table 2). FluGen-H3N2-V003 is a Phase I study in adults, testing the new high-yield Sing16 M2SR vaccine and a new high-yield Bris10 M2SR vaccine, whereas NIAD is a Phase I study using Bris10 M2SR in a paediatric population of 9-17 years old.
136. Further information regarding the previous clinical trials of the GMO vaccine is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.
137. Together, these results indicate that M2SR based vaccines are safe and generally well tolerated.

4.2.8 Shedding of virus inoculum from trial participants

138. During the First-In-Human trial of Bris10 M2SR in healthy adults (FLUGEN – H3N2-V001, see Table 2), nasal swabs were collected from all 96 trial participants on the day prior to dosing and subsequently on days 1, 2, 3 and 7 post administration. The nasal swabs were tested for virus shedding using both plaque assays using permissive cells and real-time PCR. Infectious viral particles were not able to be recovered from nasal swabs on any day post-vaccination (limit of detection 1000 TCID₅₀/mL), suggesting that Bris10 M2SR is rapidly cleared by the host immune system within 1 day post administration. In contrast, virus RNA was able to be detected using PCR in a dose-dependent manner on day 1 post vaccination. By day 7 post administration, viral RNA levels were below the limit of detection (1900 RNA copies/mL, equivalent to 3 TCID₅₀/mL). It is important to note that PCR tests do not discriminate between viable infectious particles and residual virus RNA derived from non-viable particles. Based on these data, the high-yield Sing2016 M2SR is also expected to be rapidly cleared by the immune system from trial participants with minimal shedding of the virus inoculum.
139. The applicant advised that the intranasally administered vaccine inoculum is expected to be absorbed rapidly into the upper respiratory tract following administration. Experiments using radio-labelled albumin as a vaccine surrogate to investigate the absorption of intranasally delivered vaccines demonstrated that the nasal spray was absorbed with halftimes of clearance ranging from 40-60 minutes, with a mean time of 50 minutes (Bryant et al., 1999).

4.2.9 Transmissibility

140. Transmissibility of M2SR based vaccines has not been investigated in humans. However, human to human transmission has been investigated for the commercially approved, live genetically modified attenuated influenza vaccine, FluMist (see [DIR 137 RARMP](#)). The FluMist vaccine used in the study was composed of three cold-adapted, temperature sensitive, attenuated influenza

virus strains that are able to replicate and shed progeny virions at low levels in humans. A deliberate transmission study of the FluMist vaccine was conducted with young children, 9 to 36 months of age, at day care centers (Vesikari et al., 2006). Shedding of progeny virions was detected by culturing of virions from nasal swabs collected at several time points. Vaccinated children were generally observed to shed FluMist virions between days 1 and 12 post vaccination and in some cases up to 21 days post administration. Despite the 21 day shedding period, the observed transmission rates were very low. When two possible unconfirmed transmission events were included in the analysis as confirmed cases, transmission rates using the Greenwood transmission model resulted in transmission rates of 1.75% with an upper limit of the 90% confidence interval of 8.05%. When the Reed-Frost transmission model was used, the upper bound of the 95% confidence interval for the estimated transmission rate to a child in a contact group with a single vaccinated child was 3.7%.

141. As Sing2016 M2SR is replication incompetent, and virions from the initial received inoculum are shed for a short period of time, transmission rates of the GMO would be expected to be orders of magnitude lower than those of the replication competent FluMist vaccine.
142. Further information regarding shedding and transmissibility is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

Section 5 Receiving environment

143. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes the presence of species susceptible to the GMO, the presence of the parent organism and related viral species, and environmental characteristics that may influence the likelihood of the GMOs spreading or persisting outside the site of release, or the harm they may cause.

5.1 Trial site

144. The intended primary receiving environment would be the nose, nasal turbinates and nasopharynx of trial participants, to be delivered via a nasal sprayer as an aerosol.
145. The secondary receiving environment would be the room and the clinical trial site where the GMO is dispensed, administered and waste disposed of. All clinical sites involved in the study would be equipped to handle infectious agents and procedures would be conducted in accordance with institutional policies based on Standard Precautions for handling potentially infectious substances and the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council, 2019).
146. The principal route by which the GMO may enter the wider environment is by sneezing or shedding of the initial viral inoculum from vaccinated trial participants once they leave the clinical trial site and return home. The tertiary receiving environment includes the trial participants' homes and any places they visit during the period when the GMO is shedding.

5.2 Related viral species in the receiving environment

147. Human Influenza A and influenza B viruses are endemic in Australia but their levels follow an annual pattern. Infections increase noticeably in May, peak between mid-July and mid-August, and subside in October or November.

148. Avian influenza viruses are not endemic in Australia and are not expected to become endemic in the long term, as Australia is not on the migratory flight path of water birds (ducks, geese and swans) that act as a reservoir for the disease (OCVO, 2010).
149. Swine influenza viruses have been detected in Australian pig populations, with isolated cases of human infection with swine-origin influenza viruses reported in people who work on pig farms, and one case of an adolescent acquiring swine influenza after attending an agricultural fair in September 2018 (Animal Health Australia, 2018; Smith et al., 2019; Deng et al., 2020).
150. Both canine and equine influenza viruses are not present in the Australian environment and biosecurity measures have been put in place to manage the risk of these influenza viruses being brought into Australia via imported dogs or horses. Furthermore, there is no evidence to date to suggest that canine or equine influenza viruses can infect humans (Paillot and El-Hage, 2016; Department of Agriculture, 2019).
151. Influenza viruses are part of the *Orthomyxoviridae* family, which is characterised by viruses with a segmented, negative-sense, single-stranded RNA genome. As there is no DNA intermediate, this family of viruses cannot integrate into the DNA genome of the host. The single-stranded RNA segments are not thought to be able to undergo homologous recombination. The segmented genome allows horizontal gene transfer through reassortment.
152. Reassortment only occurs with influenza viruses of the same type. Therefore influenza A virus and influenza B virus do not reassort with each other, with influenza C virus or with other *Orthomyxoviridae*. This is attributed in part to type specific virus packaging signals which are required for incorporation of a complete set of the 8 genomic RNA segments into virus particles (Baker et al., 2014; White et al., 2019) (Baker 2014, White 2019). Incompatibilities between the polymerase sub unit proteins between influenza A and B viruses may also contribute to the lack of reassortment between different influenza types (Wunderlich et al., 2010).

5.3 Similar genetic material in the environment

153. The parent organism, PR8, has been used worldwide in the manufacturing of GM flu vaccines. As a result, these GM flu vaccines have been used around the world, including in Australia. The PR8 vector backbone is highly similar to the high-yield M2SR backbone.
154. Therefore, the genetic material from the PR8 parent organism is already present in the Australian environment.
155. The haemagglutinin and neuraminidase segments introduced into the Sing2016 M2SR vaccine would be expected to be widespread in the environment, as they are derived from a target strain predicted to be circulating in human populations by the WHO's Global Influenza Surveillance and Response System for the 2018-2019 flu season.
156. All of the genes and genomic RNA segments in the GM vaccine, except for the M2 gene, would be highly similar or the same as those present in other naturally occurring influenza viruses.

5.4 Alternate hosts

157. Influenza viruses are obligate parasites, which cannot replicate outside a host as they depend on the host's proteins for many replicative processes. Influenza viruses are generally host specific.

158. Dogs and cats are able to be infected with human influenza viruses, with limited onward transmission due to differences in the sialic acid receptor (Borland et al., 2020). Guinea pigs and ferrets are the species most susceptible to wild-type human influenza as they both have similar sialic acid receptor types and distributions to humans (Bouvier and Lowen, 2010; Sun et al., 2010; Enkirch and von Messling, 2015), but are unlikely to be infected through shedding of the initial inoculum from an infected person. They are kept as pets but are neither farmed nor present in large numbers in Australia. Native birds and seals are less susceptible to wild-type human influenza viruses (see [DIR 137 RARMP](#), Chapter 1, Section 4.9), and therefore are unlikely to be infected through shedding of the vaccine inoculum.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

159. Neither the proposed GM flu vaccine Sing2016 M2SR, nor any other M2SR based flu vaccine has been previously trialled in Australia. Approval for dealings with and use of Sing2016 M2SR would be required from the OGTR and the TGA, respectively. Import of the vaccine would also require a permit from DAWE.

160. However, the Regulator has previously issued DIR licences for similar dealings with other genetically modified influenza vaccines. The Regulator issued a DIR licence ([DIR 137](#)) for the commercial supply of FluMist flu vaccines. Furthermore, the FluMist Quadrivalent influenza virus vaccine nasal spray developed by AstraZeneca Pty Ltd has been assessed and approved for use in Australia by the TGA and has been registered on the ARTG (ARTG ID: AUST R 244892) since 18 October 2016.

161. Additionally, the Regulator has approved clinical trials using GM CodaVax or other SAVE flu vaccines under a clinical trial DIR licence ([DIR 144](#)).

6.2 International approvals and experience

162. Four clinical trials have been approved overseas using M2SR based vaccines (Table 2).

Table 2. Overseas approvals for clinical trials using M2SR-based vaccines

Study Number	Phase	Vaccines tested	Participant numbers	Countries	ClinicalTrials.gov or Eudra CT Identifiers
FLUGEN – H3N2-V001	I	Bris10 M2SR	96	United States	NCT02822105
FLUGEN-H3N2-V002	Ila	Bris10 M2SR	99	Belgium	2017-004971-30*
FLUGEN-H3N2-V003	I	Bris10 M2SR Sing16 M2SR	recruiting, estimated enrolment 250	United States	NCT03999554
NIAID**	I	Bris10 M2SR	43 enrolled	United States	NCT03553940

*European Union Drug Regulating Authorities Clinical Trials (Eudra CT) database identifier

** National Institute of Allergy and Infectious Diseases (NIAID)

163. While M2SR vaccines have not been commercially approved in any country to date, other live, attenuated GM vaccines have been approved for preventing influenza infection overseas.
164. FluMist vaccines have been approved overseas for commercial use in the USA, Canada and in the EU. Both trivalent and quadrivalent forms of the vaccine have been approved. Quadrivalent versions of the vaccines were released after the WHO recommended a second influenza B virus strain for targeting by vaccines.
165. As shown in Table 3, for each of the three jurisdictions where they are commercially available, the seasonal GM flu vaccines have been assessed twice, one for the trivalent vaccine and once for the quadrivalent vaccine.

Table 3. Overseas marketing approvals for GM flu vaccines

Released in*	Jurisdiction	Vaccine type	Trade Name
2003/2004	USA	trivalent	FluMist
2012/2013	EU	trivalent	Fluenz**
2010/2011	Canada	trivalent	FluMist
2013/2014	USA	quadrivalent	FluMist Quadrivalent
2014/2015	Canada	quadrivalent	FluMist Quadrivalent
2014/2015	EU	quadrivalent	Fluenz Tetra**

*Northern hemisphere influenza season

**FluMist vaccines are marketed as Fluenz in the EU

Chapter 2 Risk Assessment

Section 1 Introduction

166. 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 4). 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.

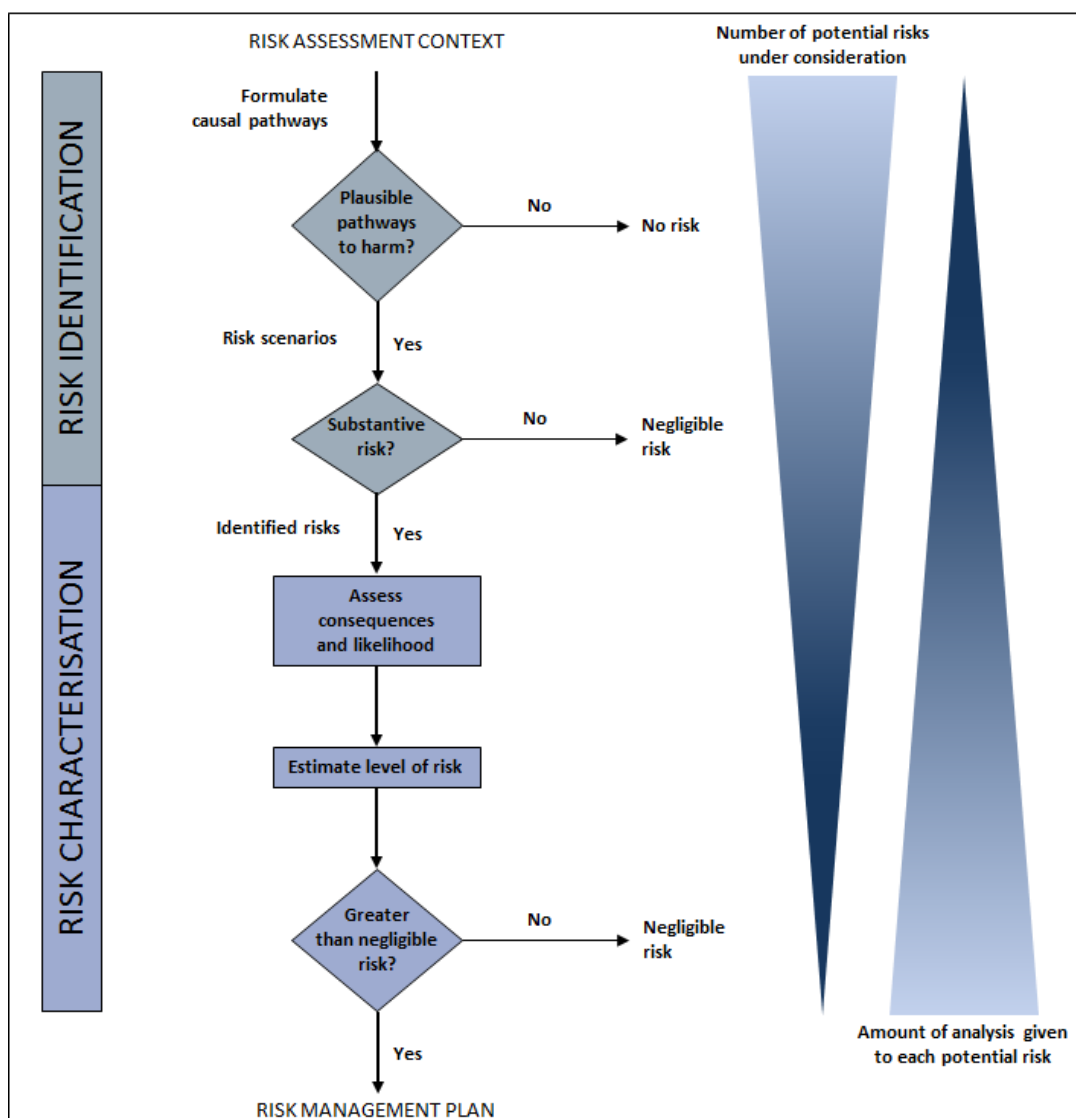


Figure 4. The risk assessment process

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

168. 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 causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios.

169. 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 long or short term, do not advance in the risk assessment process (Figure 4), i.e. the risk is considered no greater than negligible.

170. 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

171. Postulated risk scenarios are comprised of three components (Figure 5):

- i. the source of potential harm (risk source)
- ii. a plausible causal linkage to potential harm (causal pathway), and
- iii. potential harm to people or the environment.

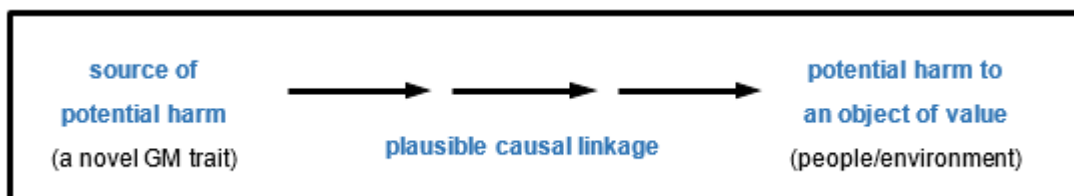


Figure 5. Components of a risk scenario

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

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

2.1 Risk source

173. The parent organism of the GMO is the respiratory pathogen, influenza virus A. Details on the pathogenicity and transmissibility of Influenza A virus is given in Chapter 1. Infection is generally the result of inhalation of aerosol droplets containing the virus or of mucosal exposure to contaminated surfaces. Disease symptoms include runny nose, fatigue, fever, cough and a sore throat and in some cases influenza infection can be fatal.

174. Infection with influenza viruses does not result in latent infection or integration into the host genome, and this will not be considered further.

175. Toxicity and allergenicity of the introduced genes and their protein products were not directly considered, but are taken into account in the context of their contribution to ill health.

176. Potential sources of harm 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. Unintended effects can arise through horizontal gene transfer (HGT), the stable transfer of genetic material from one organism to another without reproduction. All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. A gene transferred through HGT could confer a novel trait to the recipient organism. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism. Reassortment, which may be considered to be a mechanism of HGT, is a frequent source of novel influenza viruses.
177. All of the genes and genomic segments in the replication incompetent GMO would be derived from existing non-GM influenza A strains, except for genetic modifications to the M2 gene to render it non-functional (chapter 1 section 4.1.2) and the high yield changes located across multiple genome segments as described in chapter 1 section 4.1.3. These changes increase replication of the GMO in permissive cells expressing M2 protein and cause similar viruses with some of the same changes to have slightly increased replication in mice (chapter 1 sections 4.2.1 and 4.2.2). Potential risks from reassortment between the GMO and naturally occurring human influenza viruses are considered below.
178. Reassortment between different types of influenza viruses (e.g. A and B) does not occur and will not be considered further. Influenza viruses do not undergo homologous recombination as they have single-stranded RNA genomes, and this will not be considered further. Reassortment between the GMO and a zoonotic influenza virus will not be considered further due to the unlikely occurrence of a co-infection and the presence of natural barriers to reassortment between divergent influenza viruses.

2.2 Causal pathway

179. The following factors are taken into account when postulating plausible causal pathways to potential harm:
- the proposed dealings
 - the proposed limits including extent and scale of the proposed dealings
 - the proposed controls to limit the spread and persistence of the GMOs
 - routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
 - potential effects of the introduced gene(s) and gene product(s) on the properties of the organism
 - potential exposure of other organisms to the introduced gene(s) and gene product(s) from other sources in the environment
 - potential exposure of other organisms to the GMOs in the environment
 - the environment at the site(s) of the trial
 - spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential)
 - environmental stability of the organism (tolerance to temperature, UV irradiation and humidity)
 - gene transfer by horizontal gene transfer
 - unauthorised activities, and
 - practices during and after administration of the GMOs

180. Although all of these factors are taken into account, some are not included in the risk scenarios below as they may have been considered in previous RARMPs and a plausible pathway to harm could not be identified.

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

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

2.3 Potential harm

183. In addition, the following factors are taken into account when postulating relevant risk scenarios for this licence application:

- harm to the health of people or desirable organisms, including disease in humans or animals or adverse immune response
- the potential for establishment of a novel virus in the environment

2.4 Postulated risk scenarios

184. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 4 and examined in detail in sections 2.4.1– 2.4.3 (this chapter).

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

Table 4. Summary of risk scenarios from the proposed dealings with the GMOs

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	GM flu vaccine	Exposure of persons to the GMO during <ul style="list-style-type: none"> a) administration of the GMO b) transport or storage of the GMO c) disposal of the GMO; or d) collection, transport or analysis of biological samples from participants containing the GMO via aerosols, fomites, contact with abraded skin or mucous membranes <div style="text-align: center;">↓</div>	Ill health, mild flu-like symptoms	No	<ul style="list-style-type: none"> Only trained and/or experienced personnel would conduct dealings with the GMO, using personal protective equipment to minimise potential exposure Import and transport of the GMO would be in accordance with IATA UN 3373 and/or the Regulator's <i>Guidelines for Transport, Storage and Disposal of GMOs</i> GMOs and contaminated waste would be disposed of as infectious clinical waste The dose received through accidental exposure would be substantially less than that administered to trial participants and would not be sufficient for immunisation of exposed persons

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
		<p>Transduction of cells</p> <p>↓</p> <p>Expression of GM flu vaccine proteins</p>			<ul style="list-style-type: none"> The GMO is replication incompetent
2	GM flu vaccine	<p>Inoculation of a trial participant with the GMO</p> <p>↓</p> <p>Trial participant discharges unincorporated inoculum or sheds GMO progeny</p> <p>↓</p> <p>Exposure of other people (e.g. household contacts, including at risk people and pregnant women) or animals via:</p> <ul style="list-style-type: none"> - direct contact with the trial participant; - exposure to aerosolised secretions (e.g. from sneezing); or - contact with GMO contaminated items (e.g. items the trial participant has touched, or contaminated tissues); <p>↓</p> <p>Transduction of cells</p> <p>↓</p> <p>Expression of GM flu vaccine proteins</p>	Ill health, mild flu-like symptoms	No	<ul style="list-style-type: none"> The GMO inoculum administered to participants is expected to be discharged for a short time period Viral titres shed by trial participants are likely to be far lower than originally administered to them, as the GMO is replication incompetent The GMO would not be expected to regain replication competence in immunocompromised hosts
3	GM flu vaccine	<p>Trial participant inoculated with the GMO is infected with another influenza virus (contemporary circulating influenza virus)</p> <p>↓</p> <p>Both viruses co-infect the same host cell</p> <p>↓</p> <p>GMO and the wild influenza virus reassort</p> <p>↓</p> <p>Replication competent reassortant GMO virus with functional M2 protein</p> <p>↓</p> <p>Reassortant infects host and replicates</p> <p>↓</p> <p>Establishment of viral infection in host</p> <p>↓</p> <p>Reassortant virus shed</p> <p>↓</p> <p>Reassortant virus transmitted and infects other hosts</p>	Disease in humans	No	<ul style="list-style-type: none"> Co-infection of trial participants is unlikely, as only healthy children would be inoculated with the GMO and inoculations would only occur outside of the influenza season There is only a short temporal window when co-infection would be able to occur The GMO is expected to be absorbed into the upper respiratory tract within two hours The GMO is only expected to be present in the trial participant for a short time before being cleared by the immune system Reassortants that may be more virulent are unlikely to occur.

2.4.1 Risk scenario 1

Risk source	GM vaccine
Causal pathway	<p>Exposure of persons to the GMO during</p> <ul style="list-style-type: none"> a) administration the GMO b) disposal of the GMO c) transport or storage of the GMO; or d) collection, transport or analysis of biological samples from participants containing the GMO <p>via aerosols, fomites, contact with abraded skin or mucous membranes</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">transduction of host cells</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of GM vaccine proteins</p>
Potential harm	Ill health, mild flu-like symptoms

Risk source

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

Causal Pathway

187. Influenza viruses can be transmitted via aerosol droplets generated when an infected person coughs, sneezes or talks. They can also be transmitted when contaminated surfaces, such as hands or tissues, make contact with mucous membranes.

188. There are a number of additional ways that people may be exposed to the GM influenza vaccine while undertaking dealings as part of this trial.

Exposure during administration of the GMO via aerosols

189. As discussed in chapter 1, section 2.3.5 the GM investigational product would be administered to trial participants using nasal sprayers, which release the GMO as a fine spray into the nasal cavity. During these dealings, there is a potential risk of exposure to people involved in the clinical trial via aerosols generated during use of the nasal sprayer. There is a risk that the trial participants, due to their young age, may be refractory to treatment, resulting in the nasal spray being sprayed into the environment and onto nearby people in the vicinity.

190. Controls proposed by the applicant include use of medically trained clinical staff i.e. nurses and doctors provided GMO specific training and study procedure training (chapter 1 section 2.3.10). In addition, clinical trial staff administering the GMO would wear protective clothing including gown and gloves.

191. The applicant has also proposed contingency plans in case of spills, eye contact or skin contact with the GMO (chapter 1 section 2.3.11). These proposed procedures would help to mitigate any effects of the GMO to potentially exposed persons.

192. All personnel working in settings where healthcare is provided are required to comply with the standard precautions for working with potentially infectious material, as described in the [Australian Guidelines for the Prevention and Control of Infection in Healthcare \(2019\)](#).

Compliance with these behavioural practices at clinical trial sites will also limit and control exposure of people to the GMO.

193. Parents, guardians or caregivers may also be inadvertently exposed to the GMO during administration. Caregivers would only be expected to be exposed to low levels of the GMO during administration to the trial participant and this is not expected to result in any negative effects or ill-health in caregivers. Clinical trial staff administering the GMO would be expected to be potentially exposed while delivering the doses to trial participants multiple times, while caregivers would only potentially be exposed when their child is being administered the GMO. Therefore while use of protective clothing is planned for clinical staff, no protective clothing is proposed for caregivers to wear.

194. The above mentioned limits and controls would minimise the potential exposure of people to the GMOs via aerosols during administration of the GMO.

Exposure during transport or storage of the GMO

195. If the GM investigational product was unintentionally spilt or lost during transport or storage, this could result in exposure to people in the area, as aerosol droplets could be formed, leading to aerosol contact with eyes or mucous membranes and subsequent infection with the GMO.

196. As described in chapter 1 section 2.3.4 the GM investigational product would be imported and transported within Australia according to IATA UN 3373, with triple containment and appropriate labelling.

197. The GM investigational product would be labelled and double-contained for the purposes of transport and storage at clinical trial sites (Chapter 1 sections 2.3.5 and 2.3.8) and every primary container of the GM investigational product would be accounted for, minimizing the risk of loss of GMOs (chapter 1 section 2.3.12).

198. The import, transport and storage procedures proposed by the applicant meet the requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* and would limit and control risks of exposure due to spills of the GMO during these dealings.

Exposure by contact with contaminated materials/during disposal of the GMO

199. If people inadvertently had contact with materials or surfaces contaminated with the GMO, they could be infected with the GM virus through hand to mouth transmission of the virus, or through the generation of aerosols. Exposure could occur during disposal of the GM investigational product or materials contaminated with the GMO.

200. The applicant has proposed that all unused GM investigational product and waste contaminated with the GMO would be placed in clinical waste containers labelled to indicate that they contain GMOs and disposed of as infectious clinical waste by suitably experienced commercial waste contractors (chapter 1 section 2.3.9). Laundry contaminated with the GMO would also be treated by suitably experienced waste contractors using procedures suitable for infectious substances.

201. The proposed disposal and decontamination procedures would minimise and control risks associated with conducting these dealings with the GMOs.

Exposure during collection, transport or analysis of biological samples from participants containing the GMO

202. The applicant has proposed that samples would be collected from trial participants after administration of the GMO. Personnel collecting samples from trial participants, transporting or analysing the samples could be exposed to GMOs present in the samples via needle stick/sharps injury, aerosols or fomites.
203. Samples collected from the trial participants for the purposes of the clinical trial would be collected by medically trained staff, using standard precautions for the prevention and control of infection in healthcare (chapter 1 section 1.1). This would minimise potential exposure if any GMO was present in the sample.
204. The applicant advised that samples collected from trial participants may be transported to third-party testing laboratories in Australia, or exported to overseas laboratories for analysis and labelled as per UN 3373. As discussed above, the packaging requirements under UN 3373 would limit and control risks associated from transport of biological samples potentially containing the GMOs.
205. Analysis of participant samples in Australia would be conducted by personnel in analytical or pathology laboratories who are trained and experienced at handling biological samples that may contain other, more dangerous human pathogens. The National Pathology Accreditation Advisory Council (NPAAC) is responsible for developing standards and guidelines for pathology practices which include safety precautions for workers exposed to infectious pathogens (chapter 1 section 1.1). Participant samples would be analysed and stored as clinical samples by appropriate pathology services. Waste associated with participant samples would be treated as clinical waste.
206. Additionally, the GMO is expected to be rapidly cleared by the immune system in trial participants, within one day post administration, based on previously published studies in adults using similar M2SR-based GMOs (chapter 1 section 4.2.8).
207. Taken together, the behavioural requirements of healthcare professionals and personnel analysing biological samples and IATA transport requirements for biological samples would be expected to control and limit any potential exposure to the GMO through patient samples, if any GMOs were indeed present.

Potential harm

208. If people are exposed to the GMOs via needle stick/sharps injury, aerosols, fomites or other GMO contaminated waste, they could suffer from mild influenza symptoms for a short period of time. It is highly unlikely, that exposed people may experience an adverse immune response to vaccination. No adverse immune responses have been observed in clinical studies to date.
209. Any dose received through accidental exposure would be substantially less than that administered to trial participants and would not be expected to result in infection as the dose would be much lower than the immunizing dose. In addition, the GMO is replication incompetent and is expected to be rapidly cleared by the immune response. The minimal exposure and transient nature of infection would be expected to result in very mild, or negligible symptoms and would also minimise the potential for an adverse immune response to the vaccine.

Conclusion

210. Risk Scenario 1 is not identified as a substantive risk because exposure is limited by the applicants proposed limits and controls and by the mandatory use of standard precautions for

working with potentially infectious material in all Australian healthcare facilities. The GMOs are replication incompetent and are not expected to cause ill health or an adverse immune response in people who are incidentally exposed. Therefore this risk could not be greater than *negligible* and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GM vaccine
Causal pathway	<p style="text-align: center;">Trial participant inoculated with GMOs</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Trial participant discharges unincorporated inoculum or sheds GMO progeny</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of other people (e.g. household contacts, including at risk people and pregnant women) or animals via:</p> <p style="text-align: center;">a) direct contact with the trial participant;</p> <p style="text-align: center;">b) exposure to aerosolized secretions (e.g. sneezing); or</p> <p style="text-align: center;">c) contact with GMO contaminated items (e.g. items the trial participant has touched, or contaminated tissues)</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Transduction of cells</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Expression of GM flu vaccine</p>
Potential harm	Ill health, mild flu-like symptoms

Risk source

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

Causal Pathway

Trial participant discharges unincorporated inoculum or sheds GMO progeny and exposes other people or animals to the GMO

212. If trial participants discharge unincorporated inoculum or shed GMO progeny, they could contaminate surfaces with the GMO and/or generate aerosols containing the GMO when they cough, sneeze or talk. This could lead to infection of other people and animal hosts in the environment.

213. Trial participants are not expected to shed GMO progeny, as the GMO is replication incompetent. However, participants may discharge GMOs delivered in the original inoculum.

214. The applicant has proposed that trial participants would be administered the GMO at general treatment clinics. Trial participants would be required to remain at the clinical trial site for 30 minutes post-administration. Caregivers would be advised to appropriately dispose of tissues used at the clinical trial site and to wash hands (Chapter 1 section 2). These measures proposed by the applicant would help to minimise exposure of persons or animals to GMO inoculum potentially released from trial participants.

215. As discussed in chapter 1 sections 4.2.8 and 4.2.9 and in paragraph 206, the GMO inoculum administered to participants is expected to be quickly absorbed into the upper respiratory tract, so that discharge of GMO virions from the nasal cavity of trial participants would only occur for a short period of time, limiting potential spread of the GMO. Additionally, the GMO is expected to be rapidly cleared by the immune system within one day post administration.
216. It is expected that the intranasally administered GMO would be absorbed quickly by trial participants, with mean clearance halftimes of 50 minutes, based on experiments using radio-labelled albumin as a vaccine surrogate (chapter 1 section 4.2.8). This would further limit the time trial participants could secrete unincorporated inoculum through sneezing, coughing or talking. In addition, the amount of GMO secreted by trial participants is likely to be far lower than the dose originally administered to them, as the GMO is replication incompetent. The GMOs would not be expected to persist for a long period of time in the environment outside of a host, further limiting exposure to other people and animals in the environment (chapter 1 section 3.9).
217. Further information regarding the potential for the GM vaccine to be discharged into the environment is in a CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.
218. As discharge of the GMO inoculum from trial participants, if any, is expected to be very low and transmission is highly unlikely (chapter 1 sections 2.3.3, 4.2.8 and 4.2.9), the applicant is not proposing precautions to exclude subjects who have household or other contacts with immunosuppressive conditions. Carers would be required to take normal hygiene precautions, which would minimise interpersonal spread of the GMOs.

Potential harm

219. Low levels of GMOs would be expected to be secreted from trial participants for a short period of time, reducing exposure of other people or animals, such as pets, to the GMOs. The minimal exposure and transient nature of infection would be expected to result in very mild, or negligible symptoms and would also minimise the potential for an adverse immune response to the vaccine.
220. If other people or animal hosts with immunosuppressive conditions were exposed to and infected by the GMO, the GMO would be expected to remain replication incompetent and unable to cause disease. People or animals with immunosuppression may take longer to clear the GMO virus. Symptoms have not been investigated in immunosuppressed hosts, however, it would be expected that the GMO would be severely attenuated compared to naturally occurring, wild type influenza.

Conclusion

221. Risk scenario 2 is not identified as a substantive risk because the GMO is replication incompetent and trial participants cannot shed GMO progeny virions. Trial participants may discharge the administered GMO inoculum, however this would be expected to occur for a short period of time. Additionally, discharged GMO is unlikely to persist in the environment, or present harm through incidental exposure. The applicants proposed limits and controls would also help to minimise and control exposure to the discharged or shed GMOs. Therefore this risk could not be greater than *negligible* and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	GM vaccine
Causal pathway	<p>Trial participant inoculated with GMO is infected with another influenza virus (contemporary circulating influenza virus)</p> <p>↓</p> <p>Both viruses co-infect the same host cell</p> <p>↓</p> <p>GMO and the wild influenza virus reassort</p> <p>↓</p> <p>Replication competent reassortant virus with functional M2 protein</p> <p>↓</p> <p>Reassortant infects host and replicates</p> <p>↓</p> <p>Establishment of viral infection in host</p> <p>↓</p> <p>Reassortant virus progeny shed</p> <p>↓</p> <p>Reassortant virus transmitted and infects other hosts</p>
Potential harm	Disease in humans or animals.

Risk source

222. The sources of potential harm for this postulated risk scenario are the GM vaccine and a naturally occurring circulating influenza virus.

Causal pathway

223. For reassortment to occur, two different influenza viruses of the same type must co-infect a host cell (e.g. two influenza A viruses). If co-infection does occur, reassortment could result in viral progeny having any permutation of the possible combinations of the genomic segments from the parental viruses. Reassortants between the GMO and a circulating influenza virus could gain replication competence if they contain a functional M2 gene from the wild type influenza parent. The novel virus could then be shed from the recombination host and transmitted to other hosts in the environment.

224. Co-infection in trial participants could occur if the trial participant has an existing influenza infection at the time of GMO administration, or if the trial participant acquired an influenza infection while the GMO is present.

225. The applicant has proposed that administration of the GMO would only occur outside of the Australian peak influenza season and that only healthy children would receive the GMO. The applicant has also provided data from a previous clinical trial to support the effectiveness of these control measures. In a clinical trial conducted outside the influenza peak season, 96 trial participants were tested for influenza virus infection, prior to inoculation with a M2SR GMO. All of the 96 trial participants tested negative for influenza infection (chapter 1 section 2.3.3). Therefore, the proposed measures would be expected to limit the potential for trial participants to be co-infected with the GMO and a circulating influenza virus.

226. Furthermore, the frequency of co-infection of the same host cell with the GMO and a circulating influenza virus is expected to be lower than the frequency of co-infection between two replication competent influenza viruses. Experiments in cell culture showed that both co-infection and reassortment rates were strongly reduced when one of the viruses was rendered replication incompetent in the cell culture (chapter 1 section 3.6).
227. As discussed in chapter 1 section 3.6, co-infection of a host cell by a second influenza virus is strongly inhibited in a time-dependent manner both *in vivo* and *in vitro*. This results in a small temporal window where co-infection with two influenza viruses could occur. Together, these data suggest that there is a limited, small temporal window where co-infection with the GMO and a circulating virus could occur.
228. It is expected that the maximum permissive time period for co-infection to occur would be up to 24 hours post administration of the GMO. This is because no viable virus particles are able to be detected at 24 hours post administration of the GMO and the GMO is replication incompetent. Furthermore, no reassortants were able to be recovered from guinea pigs inoculated with two replicating influenza viruses 24 hours apart (chapter 1 section 3.6).
229. The applicant has advised that the GMO inoculum given to trial participants is expected to be rapidly cleared by the immune system, further limiting the time for potential co-infections to occur (chapter 1 section 4.2.8).
230. When mice were intranasally administered with original M2SR virus vaccines and live wild type Influenza viruses at the same time, no replication-competent viruses containing M2SR segments were observed (Y.Hatta & P.Bilsel, unpublished data, referred to in Hatta et al. (2017)). This suggests that the GMO is unlikely to reassort and gain replication competence *in vivo* when co-infection with a second replication competent virus occurs. This may in part be due to the replication incompetent nature of the GMO and reduced fitness of reassortant progeny.
231. Although all the possible permutations of reassortants between the GMO and a circulating influenza could occur, the reassortants would be expected to have differences in fitness levels. Reassortants with reduced fitness would be expected to be out-competed by the parental viruses. Recent studies in humans have suggested that there is negative selection against influenza virus reassortants and restrictions in the gene segment combinations being produced, leading to a reduction in the effective rate of reassortment (Sobel Leonard et al., 2017; Villa and Lassig, 2017).
232. The frequency of reassortment and possible genome segment combinations being produced can be reduced due to genetic divergence and genetic incompatibilities between the two 'parental' viruses. Generally, the greater the genetic divergence between the parental strains, the less likely reassortants are to be established and spread. This can be due to incompatibilities such as differences in genome packaging signals, non-functioning or sub-optimal combinations of the three polymerase genome segments PB1, PB2 and PA and haemagglutinin-neuraminidase imbalances (chapter 1 section 3.6).

Potential harm

233. Replication competent reassortants containing the M genome segment from the circulating influenza virus would be expected to be either less virulent, as virulent, or slightly more virulent than circulating influenza strains. All replication competent reassortants would be expected to be treatable with antivirals used for circulating influenza strains (chapter 1, section 3.10)

234. It would be unlikely that a replication competent reassortant incorporating multiple genome segments from the GMO, containing the high-yield changes would be generated (chapter 1 section 4.1.3). The high-yield changes are thought to act in combination to generate the increased replication phenotype, and therefore it is expected that all of the multiple genome segments containing the changes from the GMO parent would be needed for increased replication in reassortants.

235. An imbalance of haemagglutinin and neuraminidase activities would result in reduced fitness of reassortants. Therefore, it is expected that reassortants would most likely require the HA and NA genome segments to be both derived from either the GMO which is highly unlikely, or from the circulating virus, so that the haemagglutinin-neuraminidase balance remains intact (chapter 1 section 3.8).

Conclusion

236. Risk scenario 3 is not identified as a substantive risk. Co-infection of a host cell with the GMO and a natural influenza virus would be highly unlikely. Reassortants would only be expected to be slightly more virulent than reassortants generated from two circulating wild type influenza viruses if they incorporated multiple genome segments from the GMO and also contained the M genome segment from the naturally occurring influenza strain. Therefore, the risk could not be greater than *negligible* and does not warrant further detailed assessment.

Section 3 Uncertainty

237. Uncertainty is an intrinsic part of risk analysis². There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

238. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). These include:

- uncertainty about facts:
 - knowledge – data gaps, errors, small sample size, use of surrogate data
 - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
 - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
 - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

239. 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.

² A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR [website](#) or via Free call 1800 181 030.

240. As clinical trials are designed to gather data, there are generally data gaps when assessing the risks of a clinical trial application involving GMOs. However, clinical trial applications are required to be limited and controlled. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO, and thus decrease the likelihood of harm.
241. For DIR 171, uncertainty is noted in relation to biodistribution and persistence and secretion of the GMO inoculum. Existing work with similar M2SR-based vaccines in adults has demonstrated that the GMOs are quickly cleared by the immune system, as discussed in Chapter 1. While some uncertainty remains, it is unlikely that the GMOs would behave very differently in children and this was taken into account when estimating the level of risk.
242. Although there is a low level of uncertainty regarding the virulence of the GMO, there is very low likelihood of reassortment occurring due to the limits and controls in place. This was taken into account in estimating the level of risk.
243. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.
244. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs.
245. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 Risk evaluation

246. 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.
247. Factors used to determine which risks need treatment may include:
- risk criteria
 - level of risk
 - uncertainty associated with risk characterisation
 - interactions between substantive risks.
248. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the proposed clinical trial sites, limits and controls proposed by the applicant, and considering both the short and long term consequences, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 4 and include:
- the inability of the GMO to replicate in the absence of externally provided M2 protein
 - short expected time frame for discharge of inoculum and lack of shedding from trial participants
 - limited opportunity for the GMO to regain replication competence through reassortment with circulating influenza viruses present in the Australian environment

- influenza virus survival outside of a host is limited to short periods, and it is susceptible to common chemical decontaminants
- suitability of the limits and controls proposed by the applicant.

249. Therefore, risks to the health and safety of people, or the environment, from the proposed clinical trial of the GMO into the environment are considered to be negligible. The Risk Analysis Framework, which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed clinical trial do not pose a significant risk to either people or the environment.³

³ As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. The Regulator has allowed 5 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 Risk management plan

Section 1 Background

250. 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.

251. 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.

252. 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.

253. 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.

254. Licence conditions are discussed and summarised in this Chapter and listed in detail in the licence.

Section 2 Risk treatment measures for substantive risks

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

Section 3 General risk management

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

3.1 Limits and controls on the clinical trial

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

3.1.1 *Consideration of limits and controls proposed by Clinical Network Services Pty Ltd*

258. The proposed clinical trials would involve a maximum of 240 participants within Australia, and most dealings with the GMOs would take place in medical facilities such as clinical trial units, hospitals and analytical laboratory facilities. Activities that would occur outside of medical facilities include transport, storage and disposal of the GMOs (risk scenario 1). The applicant has proposed to complete the study within 3 years of commencement, and inoculation of trial participants would only occur outside of the Australian peak influenza season (risk scenario 3). Conditions maintaining the risk context and proposed limits of the trial such as the maximum number of trial participants, duration of the study and the permitted timing of vaccinations have been included in the licence.

259. The applicant advised that import and transport of the GM investigational product and waste containing the GM investigational product would be in accordance with relevant International Air Transport Association requirements and/or the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols for the handling of GMOs to minimise exposure to the GMOs. Once at the Clinical trial site, the GM investigational product would be triple contained, with access restricted to appropriately trained personnel. The licence requires that transport and storage of the GM investigational product and waste contaminated with the GM investigated product be in accordance with these Guidelines. These measures would limit the exposure of people and the environment to the GMOs (risk scenario 1).

260. The trial participants are limited to healthy children and relevant proposed inclusion and exclusion criteria are outlined in Chapter 1 section 2.3.3. The inclusion and exclusion criteria for trial participants would be subject to approval by a HREC, who would consider the safety of the individuals involved in the trial. The licence requires that trial participants inoculated with the GMOs must be healthy children (risk scenario 3). This also serves to minimise the potential for spread and persistence of the GM viruses.

261. The applicant advised that trial participants would be inoculated by nasal administration of the GMO by clinical staff in outpatient settings. The applicant has also proposed that clinical staff would wear personal protective equipment including gowns and gloves. These practices would minimise exposure of people handling and administering the GMOs (risk scenario 1) and have been included in the licence conditions.

262. Conditions are included in the licence requiring the licence holder to ensure that all GMOs, including material or waste that has been in contact with the GM investigational product are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Disposal of GMOs, other than by external service providers, must be in accordance with Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* for PC2 GM microorganisms. Licence conditions require that the licence holder must ensure that GM investigational products, or material or waste that has been in contact with the GM investigational products, transported by external service providers, is packaged to maintain containment of the GMOs and labelled to indicate that the consignment contains GMOs. This is considered satisfactory, provided that the licence holder is only permitted to engage persons who can adhere to appropriate standards to conduct the dealings, as described in paragraph 264. The licence also requires waste disposal by external service providers to be by autoclaving

or incineration. These measures would limit the exposure of people or other animals to the GMOs (risk scenario 1).

263. The applicant has proposed that trial participants and their parent(s)/caretaker(s) will be required to take normal precautions to minimise interpersonal spread of the GMOs after leaving the clinical trial site. The trial participant must remain at the trial site for 30 minutes following inoculation. Data from previous studies has shown that intranasally administered vaccines are absorbed into the upper respiratory tract, with mean clearance halftimes of 50 minutes (chapter 1 section 4.2.8). Therefore, there is only a short time window where limited discharge or shedding of the received GMO inoculum would occur and interpersonal transmission is not expected. In the very unlikely event that persons were unintentionally exposed to the GMO, they would be expected to have very mild to no symptoms for a short time period (risk scenario 2). Therefore it is not considered necessary to impose conditions in the licence requiring trial participants or their caregivers to undertake additional behavioural measures to limit or control transmission of the GMOs.
264. A standard condition is included in the licence requiring the licence holder to ensure that dealings are conducted so as to ensure containment of the GMO, not compromise the health and safety of people and minimise unintentional exposure to the GMO. A note written under the condition explains that compliance may be achieved by only engaging persons who are required to adhere to appropriate standards to conduct the dealings.
265. Other conditions included in the licence are standard conditions that state that only people authorised by the licence holder are covered by the licence, and that the licence holder must inform all people dealing with the GMOs of applicable licence conditions.
266. Further conditions have also been imposed in the licence to ensure that a Compliance Management Plan is in place for each clinical trial site before administration of the GMOs commences at that site. The Compliance Management Plan must detail how the licence holder intends to comply with the licence conditions, including listing persons responsible for site management, proposed reporting structures, staff training procedures and transport and disposal processes.

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

267. A number of licence conditions have been imposed to limit and control the proposed clinical trial, based on the above considerations. These include requirements to:
- limit the trial to vaccination of up to 240 trial participants at clinical trial sites, between June 2020 and April 2023
 - limit vaccination of trial participants to outside of the Australian peak influenza season
 - restrict access to the GM investigational product
 - ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements
 - ensure appropriate PPE is used
 - restrict personnel permitted to administer the GMO
 - requiring decontamination of the GM investigational product and materials and equipment that have been in contact with the GM investigational product at clinical trial sites using effective disinfectants or disposal using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation

- transport and store the GMO and samples from GMO-treated participants in accordance with IATA requirements and/or the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, in force at the time.

3.2 Other risk management considerations

268. 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 purposes of monitoring for compliance.

3.2.1 *Applicant suitability*

269. 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.

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

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

3.2.2 *Contingency Plans*

272. Clinical Network Services Pty Ltd 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 GMOs, including spills
- exposure of, or transmission to persons other than trial participants
- a person exposed to the GMOs developing a serious adverse response.

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

273. The persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to dealings with the GMOs, Clinical Network Services Pty Ltd 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

274. 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.

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

- identification of the Clinical trial sites where trial participants would be inoculated
- expected date of inoculation with the GMOs for each Clinical trial site
- cease of inoculation with the GMOs for each Clinical trial site

3.2.5 Monitoring for Compliance

276. 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.

277. 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.

278. 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 clinical trials or commercial release

279. Additional information has been identified that may be required to assess an application for a commercial release of the GMO, or to justify a reduction in limits and controls. This includes information and data that would address the uncertainties noted in Chapter 2 section 3.

Section 5 Conclusions of the consultation RARMP

280. The risk assessment concludes that the proposed clinical trial of the GMOs poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

281. Conditions are imposed to limit the trial to the proposed scale, location and duration, and to restrict the spread and persistence of the GMOs and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

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APPENDIX A SUMMARY OF SUBMISSIONS FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES

Advice received by the Regulator from prescribed experts, agencies and authorities⁴ on the consultation RARMP is summarised below. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Issues raised	Comment
1	<p>The Regulator should consider clarifying timeframes for clearance after administration and whether related risk management measures are warranted.</p> <p>The Regulator should further consider whether PPE is appropriate for caregivers/parents or any other people present at administration</p> <p>The Regulator should further consider risks associated with administration to children</p>	<p>The RARMP has been modified to clarify timeframes for clearance of the replication incompetent GMO from trial participants. The potential for secretion of the intranasally administered dose of GMO inoculum from trial participants leading to exposure of people and the environment is considered in risk scenario 2. No substantive risks to human health and safety or to the environment were identified as a result of the replication incompetent GMO.</p> <p>Additional text has been added to the RARMP regarding the necessity for PPE for caregivers/parents or any other people present at the time of administration. The potential for exposure of people to the GMOs via aerosols during administration of the GMO to trial participants is considered in risk scenario 1. No substantive risks to human health and safety or to the environment were identified as a result of the genetic modifications.</p> <p>The RARMP assesses the risks of a clinical trial of a live, replication incompetent GM influenza vaccine administered to children. Previous studies using the GMO overseas in adults suggest that the GMO has a good safety profile. Further studies are currently being undertaken overseas in adolescents. The proposed study has progressed to testing the safety and efficacy of the GM vaccine in children. The commercially</p>

⁴ Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Submission	Issues raised	Comment
		<p>available GM influenza vaccine FluMist was also trialed in children for safety, efficacy and transmission events. The OGTR has identified no substantial risks to human health and safety as a result of the replication incompetent GMO. All clinical trials in Australia require initial and on-going approval from a Human Research Ethics Committee (HREC). HRECs are responsible for ensuring the health and safety of trial participants, especially that of participants who belong to vulnerable groups, such as children. The imposed licence condition 23 requires that administration of the GMOs to trial participants must not commence prior to approval by a Human Research Ethics Committee (HREC).</p>
	The Regulator should further consider the risks associated with possible reassortment	<p>Additional consideration and discussion of the risks associated with possible reassortment was incorporated into risk scenario 3 of the RARMP.</p> <p>In a previous clinical trial overseas, 96 trial participants were tested for influenza infection prior to inoculation. None of the trial participants tested positive for influenza.</p> <p>Licence conditions have been imposed requiring that only healthy children are to be administered with the GM influenza vaccine.</p>
2	<p>Feedback:</p> <ul style="list-style-type: none"> • broadly supportive of application DIR-171. • The detailed material for this trial of a new Flu vaccine is comprehensive and describes both the molecular biology and RNA virus in general, and influenza virus in particular. • The genetic modifications incorporated into the vaccine have replication suppression as well as antigenic components. <p>Following a request from a Council member for two references cited in the RARMP:</p> <ul style="list-style-type: none"> • The two posters are informative, and indicate no significantly adverse reactions than seen in the placebo group. They also indicate potentially valuable clinical improvement in vaccinated individuals, in a 	Noted.

Submission	Issues raised	Comment
	proper double-blind trial. On this basis the member sees no reason to object to a trial in Australia.	
3	If the vaccine is proven to be safe and poses no threat to the greater community then the council would have no objections to its use in a trial. The Town has no objections to this trial subject to it being done in a way that is safe to both the public and the environment.	Noted.
4	The city has no comments or objections to the trial.	Noted.
5	Thank you for the opportunity to comment on the proposed clinical trial of GM influenza vaccine. The City does not have any comments relating to the proposed clinical trial.	Noted.
6	After considering the information provided within the RARMP, and that the outcome of the risk assessment concludes that the proposed trial poses negligible risk to people or to the environment, Council has no formal feedback to present to the Regulator. As an organisation we hope the trial proves fruitful in continuing to develop methods to ensure that people across the community are kept safe from the Influenza virus. We look forward to hearing how the trial progresses.	Noted.
7	Thank you for notifying the Council about this clinical trial. While these clinical matters are typically outside of the scope of Council expertise, this matter is one of interest due to the current COVID-19 situation. It is acknowledged you refer to a genetically modified (GM) influenza vaccine and not COVID-19. However, it would be of interest to receive updates, especially if the trial is likely to involve the Council or its residents.	Noted.
8	Overall, CNS's application has negligible risks to the health and safety of people and the environment. Specifically, the Government is satisfied that the measures taken to manage the short and long term risks from the proposal are adequate.	Noted.
9	The City supports vaccination and the development of new appropriate and suitably trialled vaccines to prevent the spread of	Noted.

Submission	Issues raised	Comment
	<p>preventable diseases, including for the Influenza A virus infection.</p> <p>Regarding this trial, I understand that the Summary of the RARMP concludes that the proposed trial poses negligible risk to people or the environment and that there will be a range of draft licence conditions that will limit the size, location and duration of the trial, as well as restrict the spread and persistence of the GMOs and the introduced genetic material. This is crucial, as the City wants to ensure that its residents, the wider community who visit the area and the environment are adequately protected from harm.</p> <p>We would appreciate it if you would please provide assurance to those community members who volunteer to ensure they are not at risk.</p>	
10	<p>Agrees that the attenuation and replication incompetence of this GM vaccine virus are likely to mean low or potentially negligible environmental risks.</p> <p>The RARMP would benefit from further discussion of the risk of shedding and the uncertainty and lack of data on this GMO regarding shedding duration.</p> <p>The RARMP would benefit from further discussion in the risk scenarios on potential transmission to animals considering the</p>	<p>Noted.</p> <p>The RARMP has been modified to include further discussion of the potential for shedding of the replication incompetent GMO from trial participants. Shedding data of a similar replication incompetent GMO has been collected from adults, but not from children. Therefore there is uncertainty regarding the duration of shedding of the GMO inoculum from children. Naturally occurring influenza virus generally sheds for a longer time in children than in adults. As the GMO is replication incompetent, the potentially increased shedding time would not result in an increased amount of GMO being shed in children. The potential risk of discharge of the GMO inoculum from trial participants leading to exposure of people and the environment is considered in risk scenario 2. No substantive risks to human or animal health and safety were identified as a result of the replication incompetent GMO.</p> <p>Additional text has been added to the RARMP discussing the potential for transmission of the GMO to animals, including pets.</p>

Submission	Issues raised	Comment
	uncertainty on shedding and potential altered host range due to the high yield changes.	<p>The replication incompetent GMO is highly unlikely to be transmitted to animals or other humans. A deliberate transmission study of the replication competent FluMist vaccine was conducted with young children at day care centers. Despite shedding of the FluMist vaccine from inoculated children for up to 21 days, observed transmission rates were very low (1.75% to 3.7%, depending on the transmission model used)). As the GMO under assessment is replication incompetent, transmission rates of the GMO would be expected to be orders of magnitude lower than those of the replication competent FluMist vaccine (chapter 1, section 4.2.9 of the RARMP). The host range of influenza viruses is dependent upon the HA and NA proteins. Therefore, the host range of the GMO is expected to be the same as the naturally occurring influenza strain A/Singapore/INFIMH-16-0019/2016 (H3N2), which was used as the source of the HA and NA protein sequences. The 'high-yield changes' to the GMO increase the yield of the GMO during the manufacturing process and are not present in the HA or NA sequences. These changes are not expected to alter the host range of the GMO.</p>
	The RARMP would benefit from greater clarity and discussion on potential reassortment risk in animals.	<p>Co-infection with the GMO and an animal influenza virus, followed by reassortment in the infected animal is highly unlikely to occur. Co-infection would be highly unlikely as the GMO is replication incompetent and only small amounts of the GMO would be shed for a limited period of time to infect an animal. If transmission were to occur, the amount of GMO secreted would not be sufficient for meaningful infection. In order for reassortment between two influenza viruses to occur, the two viruses would need to co-infect the same host cell. The risk of co-infection of a trial participant with two human influenza viruses was assessed in risk scenario 3 of the RARMP. The assessment concluded</p>

Submission	Issues raised	Comment
		that the risk could not be greater than negligible. The risk of a trial participant being infected with a zoonotic influenza virus at the time of, or very soon after administration of the GMO would be less than that assessed in risk scenario 3.
	The uncertainty and lack of data on the GM virus around shedding duration, transmission to animals, uncharacterised high-yield changes, potential altered virulence or host range; and the risk of reassortment and generation of more virulent progeny in animals should be discussed in more detail in the RARMP.	<p>The high-yield changes are thought to act in combination to generate the increased replication phenotype, and therefore it is expected that all of the multiple genome segments containing the changes from the GMO parent would be needed for increased replication in reassortants. It would be unlikely that a replication competent reassortant incorporating multiple genome segments from the GMO, containing all of the high-yield changes would be generated.</p> <p>If a replication competent reassortant was generated containing all of the high-yield changes from the GMO, there would be uncertainty regarding the virulence of the resultant virus. A similar GMO replicated faster in infected mice but there were no statistically significant changes in weight loss. It is unknown whether the GMO would be more virulent and potentially cause increased disease in people or in other animals.</p>
	Given the lack of data and uncertainty regarding shedding and therefore potential transmission to animals, trial participants should be instructed to avoid contact with animals (both native and domesticated), in the first 24 hours after inoculation to minimise the risk of transmission to animals.	The potential risk of discharge of the GMO inoculum from trial participants leading to exposure of animals is considered in risk scenario 2. No substantive risks to animal or human health and safety were identified as a result of the replication incompetent GMO.
11	The OGTR's proposed licence conditions and control measures are adequate to deal with issues that may arise on this Clinical trial and supported the conclusion that DIR 171 poses negligible risk of harm to human health and safety and the environment.	Noted.
12	No adverse comments were received. No objection to the issue of a licence for DIR 171.	Noted.

APPENDIX B SUMMARY OF SUBMISSIONS FROM THE PUBLIC ON THE CONSULTATION RARMP

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

Submission	Issues raised	Comment
1	The OGTR are mad. I am so worried for the world of our child.	Noted.
2	The OGTR and the Government are a dangerous disgrace. The OGTR appears to agree and approve every trial and release. Appalling! Coronavirus anyone? "If you really think the environment is less important than the economy, try holding your breath while you count your money" – Dr Guy McPherson	The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment, and that any risks posed by the dealings can be managed by imposing conditions on the trial.
3	Suggested additional condition of licence: 3.2.4. Licence holder to report the realisation of any potential risk events identified in the scenarios. That trials conducted at all test sites are conducted in locations that have appropriately pressurised (negative / positive) air conditioning systems.	The licence holder is required to notify the Regulator in the case of a serious adverse event (Condition 36(h)) or in case of loss or spill, or exposure of persons to the GMO (Condition 36(i)). Additionally, the licence holder must inform the Regulator, if they become aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or any unintended effects of the dealings authorised by the licence (Condition 36(f)). Together, these three licence conditions require the licence holder to report to the Regulator, if any of the events in the risk scenarios discussed in the RARMP were to occur. Exposure of clinical trial staff and caregivers to the GMO through aerosols was assessed in risk scenario 1. The risk assessment concluded that the proposed containment measures for administration of the GMO were sufficient for limiting exposure to the GMO via aerosols.

Submission	Issues raised	Comment
		No substantive risks to human health and safety or to the environment were identified as a result of the genetic modifications.
4	To whom it may concern, I strongly object to forced vaccines to take away our freedom of choice. I strongly object to being in a experimental trial...as influenza vaccines are not tested fully...in addition it contains toxic chemicals that is known to cause brain injuries, Cancers and other health related illnesses. I request that the act 2015 (no jab no pay) to be repealed. This is my will.	<p>The clinical trial is limited to a maximum of 240 participants over a period of 3 years and participation in the trial is voluntary. The Regulator must consider risks to human health and safety and to the environment posed by genetic modification being assessed in the application.</p> <p>No substantive risks to human health and safety or to the environment were identified as a result of the genetic modifications.</p> <p>The Regulator does not administer the <i>Family Assistance Act 1999</i> which concerns immunisation requirements for Family Tax Benefits and Childcare rebates and benefits.</p>