



Australian Government

Department of Health and Ageing

Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 097**

Limited and controlled release: Clinical trial of a
candidate vaccine against *Human respiratory syncytial
virus* and *Human parainfluenza virus type 3*

Applicant: PPD Australia Pty Ltd

January 2010

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 097) from Pharmaceutical Product Development Australia Pty Ltd (PPD) involving the limited and controlled release of genetically modified (GM) *Bovine Parainfluenza virus* type 3 into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a GMO. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the requirements of the legislation. RARMP's apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public¹.

The application

PPD have applied for a licence for an intentional release of a genetically modified (GM) vaccine for prevention of selected childhood respiratory diseases into the Australian environment on a limited scale and under controlled conditions.

The GM candidate vaccine is based on *Bovine parainfluenza virus* type 3 (bPIV3) which has been modified to contain genes from two common childhood respiratory pathogens *Human parainfluenza virus* type 3 (hPIV3) and *Human respiratory syncytial virus* (RSV). Expression of these genes is expected to elicit a protective immune response in vaccinated children. PPD will conduct a clinical trial of the GM vaccine in children to evaluate its safety and efficacy against hPIV3 and RSV.

The trial will take place in six specified hospitals in ACT, NSW, QLD, SA, VIC and WA and involves a maximum of 70 children aged 2 – 24 months. The trial is expected to be completed by March 2012.

PPD proposes a number of control measures to restrict exposure to the GM vaccine that were considered during the evaluation of this application.

Confidential Commercial Information

Some details, including vector maps and unpublished results of ongoing clinical trials, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals and current scientific knowledge and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, risk identification was used to postulate potential pathways that might lead to harm to people or the environment as a result of gene technology (risk scenarios) and determine those that warrant detailed characterisation.

Five risk scenarios were identified. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM virus; or produce unintended changes in viral characteristics. Gene transfer to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment.

Any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM virus into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. Risk management includes the preparation of a risk management plan to evaluate and treat identified risks, apply general risk management measures, and propose licence conditions.

As none of the five risk scenarios characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions are imposed to restrict exposure to the GMO and its genetic material in the environment and to limit the release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks.

The licence conditions requires PPD to **limit** the dealings to 70 trial participants at six clinical facilities between January 2010 and 31 March 2012. The **control** measures include administration of the GM vaccine by trained staff, containment provisions at the clinical site, controls to restrict exposure of the general public to the GM vaccine, destroying GM vaccine not required for further studies and transporting the GM vaccine in accordance with the Regulator's transport guidelines.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of the GM vaccine involving 70 trial participants in six clinical facilities in the ACT, NSW, QLD, SA, VIC and WA poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the release to the number of trial participants and the locations requested by the applicant as this were important considerations in establishing the context for assessing the risks.

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Abbreviations

ACT	Australian Capital Territory
the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
bPIV	<i>Bovine parainfluenza virus</i>
BRD	Bovine respiratory disease syndrome
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
CTX	Clinical trial exemption
DIR	Dealings Involving intentional Release
DNA	Deoxyribonucleic Acid
F	Fusion protein
FSANZ	Food Standard Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
HN	haemagglutinin-neuraminidase glycoprotein
hPIV	<i>Human parainfluenza virus</i>
ICH-GCP	International Conference on Harmonisation Good Clinical Practice standard
IATA	International Air Transport Authority
LGA	Local government area
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NSW	New South Wales
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
PCR	Polymerase Chain Reaction
PPD	Pharmaceutical Product Development Australia Pty Ltd
PR	Promoter
QLD	Queensland
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic acid
RSV	<i>Respiratory syncytial virus</i>
SA	South Australia
TGA	Therapeutic Goods Administration
VIC	Victoria
WA	Western Australia

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application (DIR 097) from Pharmaceutical Product Development Australia Pty Ltd (PPD). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) *Bovine parainfluenza virus* type 3.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public².

The application

PPD has applied for a licence for dealings involving the intentional release of a GM vaccine for prevention of selected childhood respiratory diseases into the Australian environment which qualifies as a limited and controlled release under section 50A of the Act.

The GM candidate vaccine is based on *Bovine parainfluenza virus* (bPIV3) which has been modified to contain genes from two common childhood respiratory pathogens, *Human parainfluenza virus* type 3 (hPIV3) and *Human respiratory syncytial virus* (RSV). Expression of these genes is expected to elicit a protective immune response in vaccinated children. PPD will conduct a clinical trial of the GM vaccine in children to evaluate its safety and efficacy against hPIV3 and RSV.

The trial will take place in six specified hospitals in ACT, NSW, QLD, SA, VIC and WA and would involve a maximum of 70 children aged 2 – 24 months. Once underway the trial is expected to be completed by 31 March 2012.

The applicant has proposed a number of control measures to restrict exposure to the GM virus that were considered during the evaluation of this application.

Confidential Commercial Information

Some details, including vector maps and unpublished results from related clinical trials have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment considered information in the application, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and

² More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

authorities (included in Appendix B of the RARMP) as well as the public (included in Appendix C of the RARMP).

The risk assessment begins with a risk scenario identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

Five risk scenarios were identified. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM virus; or produce unintended changes in its characteristics. The opportunity for gene transfer to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- limits on the number of trial participants and the locations proposed by PPD;
- suitability of controls proposed by PPD to restrict exposure to the GM virus; and
- widespread presence of the same or similar gene sequences and proteins encoded by the introduced genes in the environment.

Any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM virus into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this release **do not pose a significant risk** to either people or the environment³.

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. Risk management includes the preparation of a risk management plan to evaluate and treat identified risks, apply general risk management measures, and propose licence conditions.

As none of the five risk scenarios characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's Risk Analysis Framework defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict exposure to the GMO and its genetic material in the environment and to limit the proposed release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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 *Gene Technology Act 2000* mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Licence conditions

The Regulator has imposed a number of licence conditions including requirements to:

- limit the release to a maximum of 70 trial participants inoculated with the GM virus
- restrict exposure of at-risk individuals by specific exclusion criteria
- ensure that inoculations be performed by trained nurses and/or physicians at clinical facilities in accordance with standard universal precautions and ICH-GCP⁴
- store and transport all GM virus, including any waste or samples containing GM vaccine, in accordance with relevant regulations⁵
- dispose of all waste in accordance with standard clinical waste disposal practices

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have also been imposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

Australia's gene technology regulatory system operates as an integrated legislative framework involving the Regulator and other regulatory agencies that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁶.

TGA is responsible for human safety assessment of the participants in clinical trials. The clinical trial of the GM vaccine has been approved by TGA under the Clinical Trial Exemption (CTX) scheme. The OGTR liaised with TGA during the assessment of this licence application.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of the GM virus, or to justify a reduction in containment conditions. This would include:

- the potential shedding of GM virus from seronegative trial subjects;
- data on the GMO's capacity to infect or cause disease in animals; and
- data on the genetic stability of the GMO.

⁴ The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, guidelines for good clinical practice

⁵ OGTR *Guidelines for the Transport of Genetically Modified Organisms*, IATA Transportation Regulations

⁶ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* (OGTR 2009) available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of GM vaccine involving 70 trial participants in six clinical facilities in the ACT, NSW, QLD, SA, VIC and WA poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, conditions have been imposed to limit and control the release as these were important considerations in establishing the context for assessing the risks.

Chapter 1 Risk context

Section 1 Background

1. This chapter describes the parameters within which risks to the health and safety of people or the environment by the proposed release are assessed (Figure 1).

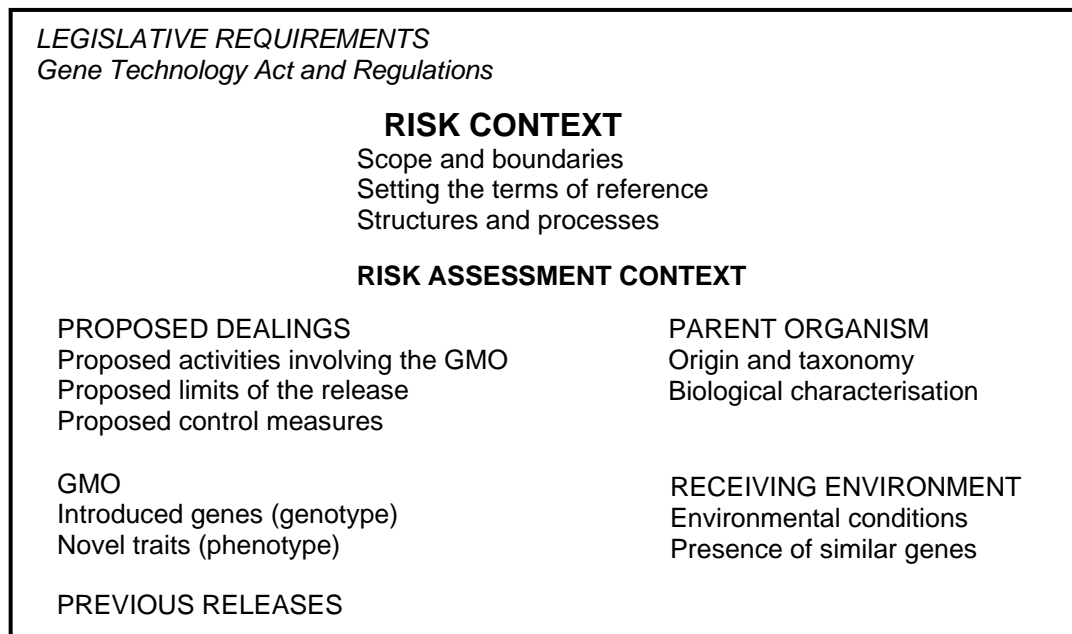


Figure 1 Components of the risk context considered during the preparation of the risk assessment

2. The risk context is developed within the framework of the *Gene Technology Act 2000* (the Act) and *Gene Technology Regulations 2001* (the Regulations) (Section 2). In addition, the Regulator's *Risk Analysis Framework* (OGTR 2009) provides general guidance on the parameters used to establish the risk context, such as, the scope and boundaries, setting the terms of reference, and the structures and processes for specific licence applications.

3. For this application, establishing the risk assessment context includes consideration of:

- scope and boundaries – interaction with other regulatory schemes (Section 3)
- the proposed dealings (Section 4)
- the parent organism (Section 5)
- the GMOs, nature and effect of the genetic modification (Section 6)
- the receiving environment (Section 7)
- previous releases of these or other GMOs relevant to this application (Section 8)

Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the Act outline the matters which the Regulator must take into account, and with whom he must consult, in preparing the RARMPs that form the basis of his decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the

applicant to conduct experiments. In addition, limits have been proposed on the size, locations and duration of the release and controls have been proposed by the applicant to restrict spread and persistence of the GMO that could lead to exposure of people and the environment to the GMO and its genetic material. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d).

Section 3 Scope and boundaries

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. 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 registered on the Australian Register of Therapeutic Goods. The Therapeutic Goods Administration (TGA) is responsible for administering the provisions of this legislation. Clinical trials usually involve the use of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, and require approval from TGA through the Clinical Trial Exemption (CTX) scheme or the Clinical Trial Notification (CTN) scheme.

9. Where the clinical trial may involve a GMO, TGA has primary regulatory responsibility for patient safety, however authorisation is also required under gene technology legislation. In order to avoid duplication of regulatory oversight the Regulator is responsible for assessing risks posed to other people who may be involved in the dealings and to the environment. In the context of this RARMP risks to trial participants will not be considered as part of the evaluation.

10. The International Conference on 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. The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States, as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). TGA has adopted the ICH-GCP in principle as Note for Guidance on Good Clinical Practice (designated CPMP/ICH/135/95). It provides overarching guidance for conducting clinical trials in Australia.

11. The NHMRC has issued the *National Statement on the Ethical Conduct in Research Involving Humans*. This document sets the Australian standard against which all research involving humans is reviewed.

12. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HRECs conduct both an ethical and a 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.

Section 4 The proposed dealings

13. Pharmaceutical Product Development Australia Pty Ltd (PPD) propose to release a vaccine based on *Bovine parainfluenza virus* type 3 (bPIV3) that has been genetically modified (GM) to contain genes from two common childhood respiratory pathogens *Human parainfluenza virus* type 3 (hPIV3) and *Human respiratory syncytial virus* (RSV), into the environment under limited and controlled conditions. Expression of these genes is expected to elicit a protective immune response in vaccinated children.

14. These dealings would form part of a worldwide clinical study “A Phase 1/2a randomised, double-blind, placebo-controlled dose-escalation study to evaluate the safety, tolerability, immunogenicity and vaccine-like viral shedding of MEDI-534, a live, attenuated intranasal vaccine against *respiratory syncytial virus* (RSV) and *parainfluenza virus* type 3 (PIV3), in healthy 6 to < 24 month-old children and in 2 month old infants”, designated MI-CP178 by the sponsor. PPD is seeking approval for dealings associated with the Australian arm of this trial of the GM vaccine.

15. The dealings involved in the proposed intentional release would include:

- importing the GMO
- conducting experiments with the GMO involving limited and controlled release into the environment
- transporting the GMO
- disposing of the GMO

The dealings would also include the possession, supply or use of the GMO for the purposes of, or in the course of, any of the dealings mentioned above. These dealings are described in more detail throughout the remainder of this Chapter.

16. Some details of the application including vector maps and unpublished results from related clinical trials have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and will be made available to the prescribed expert groups and authorities that will be consulted on this application.

4.1 The proposed activities

17. The applicant has stated that the objective of the proposed clinical trial is to investigate the safety and tolerability of multiple doses of the GM vaccine in RSV and hPIV3 seronegative⁷ children 6 to less than 24 months of age and in unscreened infants 2 months of age. Secondary objectives of the study are to investigate the immunogenicity⁸, viral shedding and genotypic stability of the GM vaccine and to investigate the incidence of serious RSV disease in vaccinated participants.

18. The GM vaccine proposed for release would be imported from the USA and transported to the central storage and distribution site at Cryosite, Lane Cove, NSW, before being transported to the clinical trial sites.

⁷ Seronegative refers to the absence of antibodies in the blood and indicates lack of exposure to the virus.

⁸ Immunogenicity is the ability of an antigen to induce an immune response.

19. Three separate inoculations of each trial participant, separated by a period of 8 weeks, would occur at clinical sites. The inoculations would involve intranasal administration of a 0.2ml dose from a pre-filled syringe.

20. All of the inoculations would be conducted by trained staff and be undertaken at the clinical facilities listed in Table 1.

Table 1 Proposed localities for release of GM vaccine

Clinical Facility	Local Government Area	Locality
Women's and Children's Hospital, Adelaide	The City of Adelaide	Adelaide, SA
Royal Children's Hospital	The City of Brisbane	Herston, Qld
The Canberra Hospital	n/a	Garran ACT
Princess Margaret Hospital for Children	City of Subiaco	Subiaco, WA
Royal Children's Hospital	Melbourne City Council	Parkville, Vic
Sydney Children's Hospital	Randwick City council	Randwick, NSW

21. Trial participants will return to the clinical facilities at 7, 12 and 28 days after inoculation and samples will be collected in order to test the level of viral shedding and genotypic stability of the GM virus. The samples will be transported to the USA for analysis.

4.2 The proposed limits and controls of the dealings

22. The release is proposed to take place at six clinical facilities located in the local government areas of the City of Adelaide SA, the Australian Capital Territory, the City of Brisbane Qld, Melbourne City Council Vic, Randwick City Council NSW, and the City of Subiaco WA, over 26 months from January 2010 to March 2012.

23. The applicant initially proposed to enrol 70 individuals in the trial – 35 of which would be inoculated with the GMO and 35 inoculated with a non GMO placebo. During the assessment process the applicant amended this proposal, requesting a larger number of trial participants to be inoculated with the GMO if required.

24. The applicant now intends to enrol 70 participants into the trial, of which up to 35 would be assigned to control groups. Over the six release sites a maximum total of 70 individuals are proposed to be inoculated with the GM vaccine.

25. The applicant has proposed a number of controls to restrict exposure to the GM vaccine and the introduced genetic material including:

- exclusion of participants that may come into contact with individuals at risk of disease from exposure to the GM virus within 28 days after each vaccination including those:
 - living in the same home or enrolled in the same classroom at day care with infants less than 6 months of age
 - in contact with a pregnant caregiver
 - in contact with a person who is immuno-compromised
 - in contact with a health care provider for immuno-compromised patients or a day care provider for infants under the age of 6 months
- clinical staff involved in the study would be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in ICH-GCP

- clinical staff involved in the handling or administration of study vaccine would be trained according to the study protocol and study specific laboratory and clinical trial material manuals
- clinical staff responsible for administering the GMO and collection of clinical specimens or clinical evaluation of study participants would be instructed to follow the World Health Organisation universal precautions for the prevention of transmission of infectious agents in healthcare settings (World Health Organisation 2007)
- the GM vaccine would be stored in an outer package in a secure location with access limited to medical staff participating in the study
- the vaccine would be transported to the clinical site according to the Regulator's *Guidelines for the transport of GMOs*
- following administration, used study vaccine syringes would be placed immediately into locked containers or sealed bags and retained for accountability
- upon reconciliation and accountability used study vaccine syringes would be destroyed at the clinical site following institutional procedures for the disposal of biohazardous material
- waste generated during the conduct of the study would be discarded into appropriate biohazard containers and disposed of at the clinical site following institutional procedures for the disposal of biohazardous material
- unused study vaccine would be returned to MedImmune's central storage depot in Australia or disposed of at the clinical site following institutional procedures for the disposal of biohazardous material.

26. Written informed consent from the trial participant's parent or guardian would be required for participation in the trial. This would be monitored by the relevant HREC.

27. The study would be monitored on a regular basis throughout the study period by a Safety Monitoring Committee, including compliance with procedures and record keeping, the study protocol, handling of the vaccine and clinical samples, collection of informed consent and safety reporting according to Ethics Committee requirements.

28. These controls and the limits outlined in Chapter 1, Section 4.2 have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.1.2.

Section 5 The parent organism

5.1 Bovine parainfluenza virus taxonomy

29. The parent organism is *Bovine parainfluenza virus* type 3, a single stranded, negative sense RNA virus of the Family *Paramyxoviridae* (ICTV 2009). The taxonomy of bPIV3 is outlined in Table 2.

Table 2 Taxonomy of Bovine Parainfluenza Virus type 3

Order	<i>Mononegavirales</i> (Single stranded, negative sense RNA viruses)
Family	<i>Paramyxoviridae</i>
Subfamily	<i>Paramyxovirinae</i>
Genus	<i>Respirovirus</i>
Species	<i>Bovine parainfluenza virus 3</i>

30. The family *Paramyxoviridae* includes two subfamilies, the *Paramyxovirinae* and the *Pneumovirinae*. Five genera comprise the subfamily *Paramyxovirinae*; *Respirovirus* in which *Bovine parainfluenza virus* type 3 and *Human parainfluenza virus* type 3 are closely related, *Morbillivirus*, which includes *Measles virus* and *Canine distemper virus*, *Henipavirus*, which includes *Hendra virus* and *Nipah virus*, *Rubulavirus*, which includes *Mumps virus*, and *Avulavirus*, which includes avian viruses. The subfamily *Pneumovirinae* is divided into two genera; *Pneumovirus* including human and *bovine respiratory syncytial virus*, and *Metapneumovirus*, including human and avian metapneumovirus. The taxonomic relationships between these subfamilies, genera and species is depicted in the phylogenetic analysis of (Nayak et al. 2008) (see Figure 2).

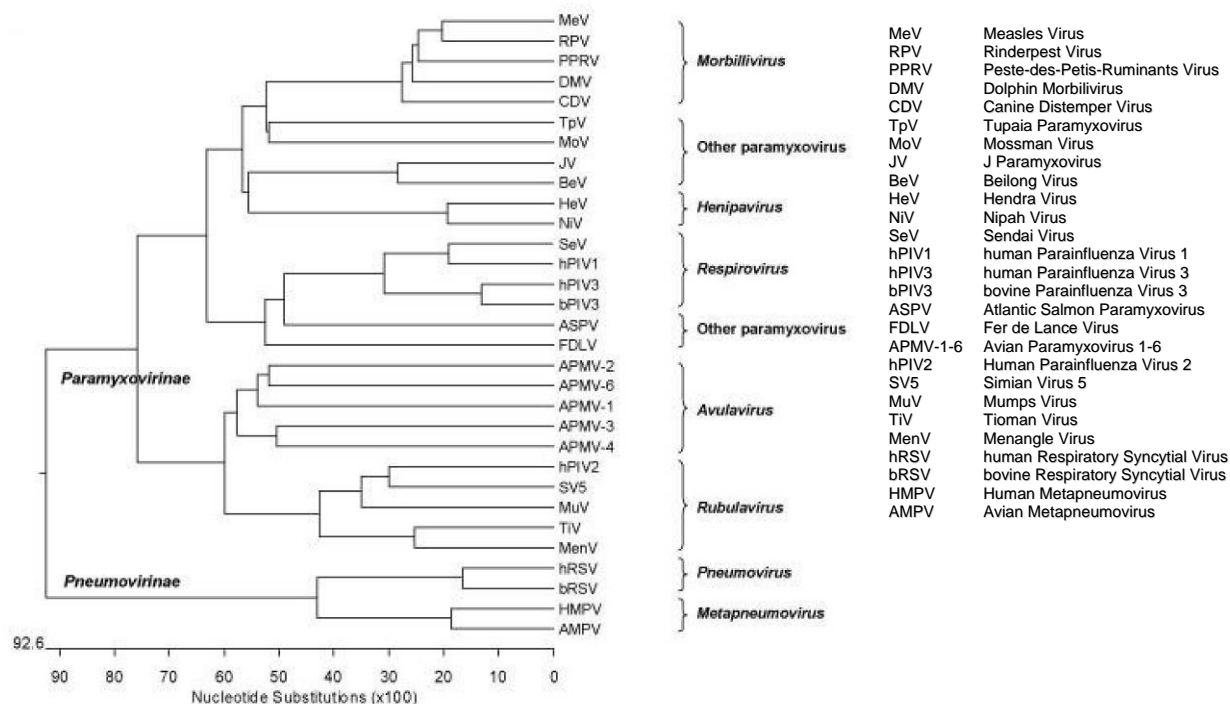


Figure 2 Phylogenetic relationships among the *Paramyxoviridae*

31. The close evolutionary relationship between bovine and human parainfluenza type 3 is reflected in overlapping antigenic profiles (Abinanti et al. 1961; Coelingh et al. 1986). *Bovine parainfluenza virus* 3 demonstrates restricted replication in human cells and has been trialed as a non GM vaccine against hPIV3, providing a degree of cross-protective immunity in the absence of significant symptoms (Wyke Coelingh et al. 1988; Clements et al. 1991; Karron et al. 1995a; Karron et al. 1996). The medical applications of bPIV3 are discussed further below.

5.2 *Bovine parainfluenza virus* genome

32. The genome of bPIV3 consists of a single strand, negative sense RNA molecule 15456 nucleotides in length, coding for six genes that are sequentially transcribed as separate mRNAs by the viral RNA dependent RNA polymerase/replicase. The genomes of bPIV3 and hPIV3 are identical in gene content and gene order. In order from the transcription start site these genes are as follows:

- NP gene which codes for the nucleocapsid protein (NP).
- P gene which codes for four proteins:
 - the Phosphoprotein (P), that forms a component of the RNA dependent RNA polymerase/replicase;

- The C protein, a small basic protein which is associated with the nucleocapsid and the polymerase complex;
- The V protein, which binds to free NP protein (not bound to the genomic RNA) and acts to inhibit RNA replication; and
- The D protein, an accessory protein.
- M gene which codes for the matrix protein (M).
- F gene which codes for the fusion protein (F).
- HN gene which codes for the haemagglutinin-neuraminidase glycoprotein (HN).
- L gene which codes for the major component of the RNA polymerase complex (L).

33. Between each gene, non-coding sequences of over 100 nucleotides contain short (approximately 10 nucleotide) consensus sequences that initiate (R1) and terminate (R2) transcription of individual genes (Suzu et al. 1987; Sakai et al. 1987).

34. Transcription of mRNAs occurs in sequence from the NP gene with the polymerase remaining on the RNA while the mRNA is polyadenylated at the R2 site and released, before initiating transcription of the following gene at the subsequent R1 site. Each time that an mRNA is released there is a certain probability that the polymerase will fall off the RNA template, and this results in a decreasing gradient of mRNA expression from the NP gene to the L gene (Chanock et al. 2001).

5.3 *Bovine parainfluenza virus life cycle*

35. The life cycle of a virus involves the transmission of infective viral particles to a host organism, recognition, attachment and entry into the host cells and then replication of viral nucleic acid and protein production, followed by assembly and release of infective virus. Viruses have co-evolved with their host species and are generally specific for that host organism and infect only certain tissue types within that organism.

36. The parainfluenza virus life cycle involves transmission from an infected host organism, via the transfer of respiratory tract secretions as aerosolized droplets, or contaminated surfaces, to the respiratory tract membranes of a susceptible host organism.

37. As the bPIV genome is single-stranded, negative-sense RNA, it must be transcribed into message-sense RNA before it can be translated into proteins. All parainfluenza viruses encode and package an RNA-dependent RNA polymerase in the virion particles to synthesise message-sense RNA (Moscona 2005).

38. Infection of a cell by bPIV is initiated by binding to the target cell via interaction of the viral receptor-binding molecule, HN, with sialic acid-containing receptor molecules on the cell surface. The viral envelope then fuses directly with the plasma membrane of the cell, mediated by the viral fusion protein (F), releasing the nucleocapsid into the cytoplasm (Moscona 2005).

39. The nucleocapsid is a helical structure consisting of the viral genomic RNA tightly associated with NP. This complex is highly resistant to RNase degradation (Coronel et al. 2001) and serves as the template both for transcription and for replication of the bPIV genome (using the viral RNA-dependent RNA polymerase) (Moscona 2005). Viral proteins are produced by the cellular protein synthesis pathways, with the HN and F envelope proteins undergoing the addition of glycosyl side chains and transport to the cell membrane. The

presence of the F protein on the surface of an infected cell may also result in fusion with a neighbouring cells and subsequent formation of a syncytium (multinucleate cell mass).

40. The replicated genome is packaged into virions incorporating NP, P, and L proteins surrounded by a shell of M proteins (Figure 3). The M protein interacts with the cytoplasmic domains of the HN and F viral glycoproteins embedded in the cell membrane to mediate budding of the virus encased in an envelope of cell membrane.

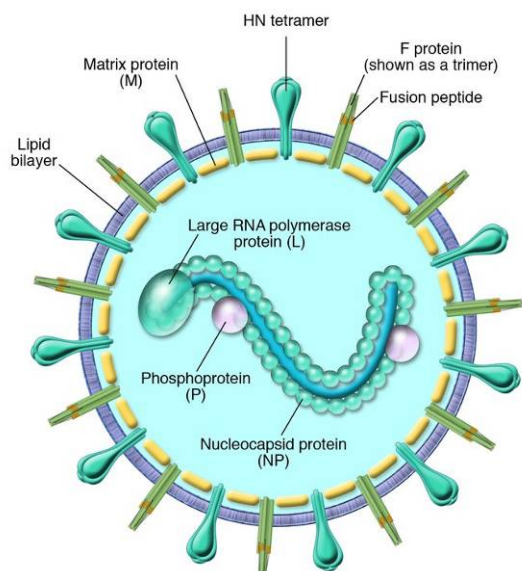


Figure 3 Schematic of the parainfluenza virion (Moscona 2005)

41. In addition to their primary role in viral replication, the C, V and D proteins of parainfluenza viruses act to inhibit interferon signalling and hence down regulate the host antiviral immune response (Komatsu et al. 2007).

5.4 *Bovine parainfluenza virus* host range

42. The host range of bPIV3 includes several mammalian species. In addition to bovine species, experimental infection is possible in sheep (St George 1969), guinea pigs, rats, ferrets (Henrickson 2003) and restricted replication is possible in humans (Karron et al. 1995a; Karron et al. 1995b) and non-human primates (van Wyke Coelingh et al. 1988).

43. Antibodies to bPIV3 have been detected in horses, goats, deer (Afshar 1969) and water buffalo (St George 1969). Phylogenetic analyses of parainfluenza 3 virus isolated from pigs and cattle suggest that these are isolates of the same virus and not distinct species-specific swine and bovine parainfluenza 3 strains (Qiao et al. 2009). While it is possible that several distinct viral strains circulate amongst domestic animals, it is more likely that bPIV3 has a broad host range and is able to infect a range of species, although these infections may not result in clinical symptoms or ongoing transmission.

5.5 Pathology of *Bovine parainfluenza virus* infection

44. The clinical presentation of bovine infections with bPIV3 can vary considerably, ranging from asymptomatic infections to severe respiratory illness (Horwood et al. 2008). In uncomplicated infection, disease is characterised by mild, rapidly resolving illness that may include the following symptoms: fever, rhinitis with nasal discharge, lacrimation, coughing and dyspnea (Jones et al. 1997).

45. Under high stress (eg. when in feedlots or during transport) bPIV3 infection is thought to render animals susceptible to secondary bacterial infection, which can lead to severe

bronchopneumonia. This multifactorial syndrome is termed bovine respiratory disease syndrome (BRD), and is one of the most significant illnesses associated with cattle in feedlots (Jones et al. 1997; Cusack et al. 2003).

46. The mild outcomes associated with natural, uncomplicated bPIV3 infection are not reflected during experimental infection. In one study (St George 1969), steers were inoculated by intratracheal intubation with 2×10^{12} median tissue culture infective dose (TCID₅₀) of bPIV3. Steers demonstrated significant symptoms including pneumonia lasting for ten days and shed virus in nasal secretions, peaking on day five after inoculation, at 10^5 TCID₅₀ (approximately 10^7 TCID₅₀ less than the inoculating dose). The increase in symptoms however may be attributable to the high inoculating dose and the mode of exposure, as intratracheal inoculation is believed to result in more severe symptoms than intranasal inoculation (Vangeel et al. 2009). Experimentally inoculated steers could infect sentinel steers housed together in close contact but this did not result in clinical symptoms in the sentinel steers. Based upon the shedding data obtained in this study, animals infected naturally would be expected to receive a much lower dose of bPIV3.

5.6 Genetic determinants of restricted replication

47. The restricted replication of bPIV3 in certain host species and particular cell types is due to requirements for specific cellular proteins. Specific surface glycoproteins must be present on host cells to enable bPIV3 HN to bind and therefore initiate an infection. Other factors that are required include:

- specific host proteases, to activate bPIV3 F proteins during release of virions; and
- specific host cell proteins that are required to interact during folding and transport of HN and F proteins.

An absence of one or more of these factors may contribute to reduced replication of bPIV3 in primate hosts (Schmidt et al. 2000).

48. Experiments involving the substitution of bPIV3 genes into hPIV3 have demonstrated that the genetic loci that determine restricted replication in primates are distributed across all six genes (Skiadopoulos et al. 2003). Substitution of the P gene, encoding P, C, V and D proteins was found to have the greatest negative effect on replication in primates *in vivo*. This is consistent with the function of the C, V and D proteins as inhibitors of interferon alpha signalling and the innate antiviral responses in the host.

49. Substitution of the bPIV3 N, F, HN, and L genes and to a lesser extent the M gene also attenuated the chimeric virus *in vivo* with respect to *Human parainfluenza virus 3*, but not *in vitro*. This suggests that interactions between the bPIV genes and primate host cell factors, rather than with hPIV3 genes, are responsible for the attenuated phenotype.

50. Furthermore, viral proteins associated with the genomic RNA, including the NP and the P, C, V and D proteins translated from the P gene are thought to block recognition of double stranded RNA by host innate antiviral defence molecules. Double stranded RNA does not occur within an uninfected cell but occurs during viral genome replication and is a marker of RNA viral infection, and thus these viral proteins act to inhibit innate host antiviral defence pathways (Skiadopoulos et al. 2003). Interactions between these viral proteins and host antiviral defence molecules may be species specific and hence contribute to determining the host range of the virus.

51. The availability of proteases for F protein activation may also be a major determinant of respiratory tissue tropism (Chanock et al. 2001).

5.7 *Bovine parainfluenza virus* environmental stability

52. Enveloped RNA viruses, such as the parainfluenza viruses, are relatively sensitive to heat, desiccation, household disinfectants and detergents. These viruses cannot not survive for extended periods outside the host organism. Studies have demonstrated that parainfluenza viruses may remain infectious for 10 hours on a wet non-absorbent surface, 2 hours on a dry non-absorbent surface, 2-4 hours on a dry absorbent surface and one hour on the skin (Brady et al. 1990).

5.8 Prevalence of infection and associated disease an Australia

53. *Bovine parainfluenza virus 3* is endemic in Australia and is ubiquitous among feedlot age cattle. On its own, infection with bPIV rarely results in significant symptoms. However, as discussed above, acute bPIV infection may suppress the normal immune response, which can leave cattle susceptible to bacterial infection that can result in multifactorial diseases such as BRD.

54. Serological surveys of Australian cattle herds have found that 77 to 94% of cattle surveyed in 252 herds were seropositive for bPIV3 (St George 1969). A subsequent study in 1991 found that of cattle entering feedlots, 57% were seropositive for bPIV3. A further 30% of cattle were found to seroconvert during their stay in the feedlots (Dunn et al. 1995). However, only 7% of cattle required treatment for BRD, suggesting that the majority of bPIV3 infections were asymptomatic.

5.9 *Bovine parainfluenza virus 3* as a vaccine against *Human parainfluenza virus 3*

55. Studies have demonstrated that bPIV3 has restricted replication in primates, causing an asymptomatic infection that induces resistance to hPIV3 (Wyke Coelingh et al. 1988; Clements et al. 1991; Karron et al. 1996),

56. Based upon animal studies, wild type bPIV3 has been trialled as a potential vaccine to protect people against hPIV3. Illness due to hPIV3 infection occurs in seronegative infants, but previously exposed adults may be infected, with replication being dampened by acquired immunity and resulting in only mild symptoms, if any. Studies in hPIV3 seropositive adults have found that intranasal inoculation with 10^6 or 10^7 TCID₅₀ of bPIV3 will result in bPIV3 replication and shedding at comparable levels to that observed in seropositive adults exposed to the same levels of hPIV3, and like hPIV3 would not result in illness (Clements et al. 1991).

57. bPIV3 is also well tolerated in seronegative children. In trials involving the intranasal inoculation of seronegative children 2-6 months (Karron et al. 1996) and 6-60 months (Karron et al. 1995a), inoculation with 10^5 TCID₅₀ bPIV3 resulted in > 90% of participants becoming infected. bPIV3 was shed for 10 days, with peak shedding rates approximately 10 fold lower than the level of inoculation. No clinical symptoms were attributed to the bPIV3 inoculation.

58. Transmissibility of the vaccine bPIV3 strain was investigated in a study where 28 seronegative children remained in a playroom setting for 4-6 hrs a day for 10 days after inoculation. Half received the vaccine and half received the placebo. No bPIV3 vaccine virus was recovered from placebo recipients although two developed an antibody response to both hPIV3 and bPIV3. Illness due to respiratory tract infections were common in both vaccine and placebo recipients and although it is possible that it was due to exposure to the virus shed by vaccine recipients, it is likely that this antibody response was due to infection with wild type hPIV3 (Karron et al. 1995a).

Section 6 The GMO, nature and effect of the genetic modification

6.1 Introduction to the GMO

59. The GM candidate vaccine virus is based on bPIV3 that has been modified to contain genes from two common childhood respiratory pathogens; *Human parainfluenza virus* type 3 (hPIV) and *Human respiratory syncytial virus* (RSV). Table 3 (below) lists the genes inserted into the parent organism.

Table 3 The genes used to alter the antigenic properties of bPIV3

Gene	Function of protein	Source	Intended purpose
hPIV3 HN	Haemagglutinin-Neuraminidase protein mediates the adsorption to, and release of virus from the host cell	hPIV3/Texas/12084/1983	Elicit an immune antibody response against hPIV3
hPIV3 F	Fusion protein mediates fusion of the viral envelope membrane with the host cell membrane	hPIV3/Texas/12084/1983	Elicit an immune antibody response against hPIV3
RSV F	Fusion protein mediates fusion of the viral envelope membrane with the host cell membrane	RSV A2 C4G (Jin et al. 1998)	Elicit an immune antibody response against RSV

60. As discussed in Chapter 1, Section 5.9, bPIV3 itself has been trialled as a live vaccine because it replicates but is not virulent in humans, is antigenically similar to hPIV3 and can provide a degree of cross protection against hPIV3. Bovine PIV3 follows the same route of infection as other paramyxoviruses that cause respiratory disease in humans, such as hPIV3 and RSV. This has made it a candidate vaccine vector to deliver hPIV3 and RSV antigens to the nasal epithelia, in order to induce appropriate and lasting immune responses without the residual virulence that may be associated with live attenuated hPIV3 or RSV (Tang et al. 2004).

61. For the parainfluenza viruses, the HN and F glycoproteins are the primary antigens that elicit a neutralising, protective antibody response and therefore the GM vaccine has been modified to replace the bPIV3 F and HN glycoproteins with the corresponding hPIV3 F and HN glycoproteins (Haller et al. 2000; Pennathur et al. 2003; Greenberg et al. 2005) (see Table 3, above).

62. Furthermore, to enable the GM vaccine to also elicit an immune response against RSV, the RSV envelope Fusion (F) glycoprotein has been inserted in such a manner to allow expression of the RSV F protein (Tang et al. 2003; Tang et al. 2004), in order to induce an immune response against RSV infection (see Table 3, above). The structure of the GM vaccine genome is represented in Figure 4 below.

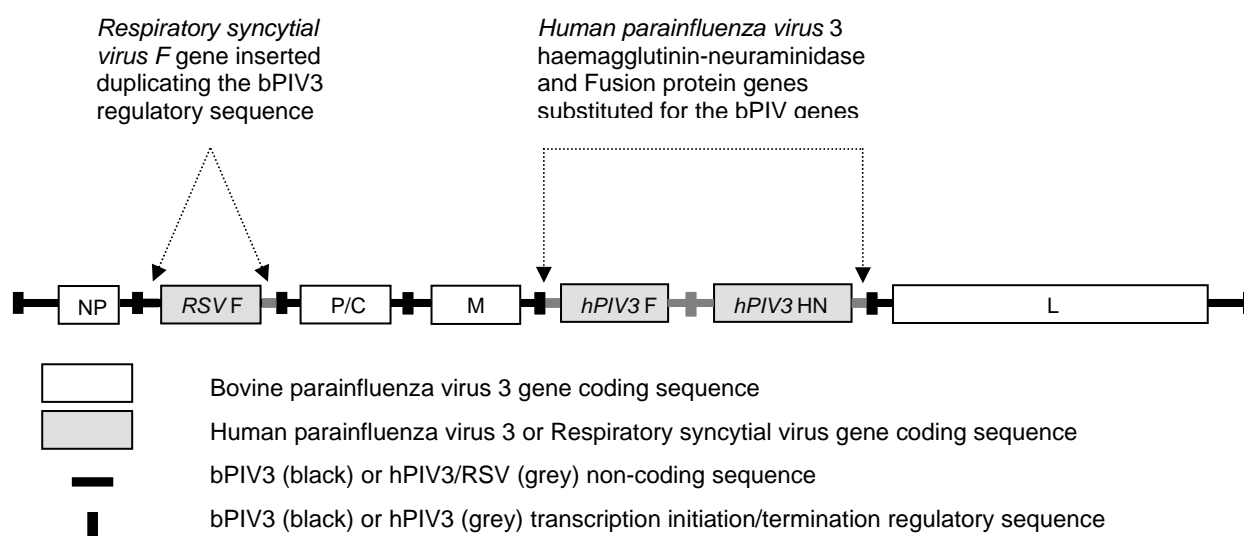


Figure 4 Structure of the GM virus vaccine genome

63. The hPIV3 and RSV genes have been inserted into the bPIV3 genome at restriction sites within the non-coding regions between genes, in a manner that preserves the R1 and R2 functional domains that control the initiation and termination of the transcription of individual genes. The hPIV3 HN and F genes together with the non-coding region that separates them, have been substituted for the homologous bPIV3 genes as a single inserted sequence.

64. The RSV F gene, together with short sections of the flanking intergenic regions, has been inserted into a restriction site within the intergenic region between the bPIV3 N and P genes.

65. The introduction of the RSV F gene may contribute to the attenuation of the GM virus by introducing a new transcription termination site close to the start of the genome at which the polymerase may be released from the RNA template. This is supported by 10 to 100 fold lower growth rate in tissue culture, however contradictory results were found *in vivo*, with no significant attenuation observed as a result of this modification following inoculation of semi-permissive Syrian Golden Hamsters (Tang et al. 2003), suggesting that rate of transcription is not the prime factor limiting viral growth *in vivo*.

6.2 Results of previous clinical trials with the GM virus

66. The GM virus has been tested in clinical trials and demonstrated an acceptable safety profile with no medically significant vaccine-related adverse events when administered to 60 healthy, seropositive adults 18-40 years of age (Tang et al. 2008).

67. A clinical trial of the GM virus in healthy, seropositive children 1-9 years of age was completed in the USA in 2008.

68. The GM virus is expected to replicate and be shed from vaccinated participants. In twenty seropositive participants, shedding was detected in one trial subject, although at a level too low to be quantified (Tang et al. 2008). Such a low level of shedding is indicative of restricted replication of the virus and is one factor that contributes to attenuation.

69. The GM virus is currently being evaluated in a worldwide study that has been approved in a number of countries (see Section 8.2). Preliminary results support ongoing studies with the GM virus and trial participants are currently being recruited in the USA.

6.3 The introduced genes, their encoded proteins and their associated effects

70. Full gene sequences have been used for the genetic modifications. The purpose of these modifications is to replace bPIV3 proteins with functionally homologous genes from hPIV3 and to add an extra homologous gene from RSV. The result of these modifications is to produce a replication competent genetically modified virus that is designed to elicit a protective immune response against the hPIV3 and RSV envelope glycoproteins.

71. The hPIV3 HN and F genes and their encoded proteins perform homologous roles to the bPIV3 genes that they replace, as discussed above. These genes may be expected to interact more efficiently with human host cells than their bPIV3 homologs and possibly reduce attenuation of the modified bPIV3 in human cells (Schmidt et al. 2000). As they are displayed on the surface of the virus the HN and F proteins are the primary antigens eliciting neutralising and protective antibodies.

72. The RSV F protein, like the parainfluenza F protein, mediates fusion of the viral envelope with the cell membrane. When translated in an infected cell it undergoes the same processing and transport as the homologous PIV3 protein, including activation by cellular proteases. When expressed on the surface of an infected cell, the RSV F protein is able to mediate fusion with a neighbouring cell to form a multi-nucleate cell mass or syncytia, providing an alternative means of viral infection of nearby cells (Collins et al. 2001).

73. Molecular variation in the F proteins of RSV strains results in differences in the severity of symptoms. In one RSV strain (line 19) the fusion protein has been identified as a virulence factor, inducing excessive mucus production, and the amino acid sequence responsible for this property has been identified (Moore et al. 2009). However the A2 C4G strain that provided the F protein gene used in the construction of the vaccine does not possess the mucogenic phenotype, and differs from the virulent strain at the amino acid sequences implicated in determining virulence. The F protein used in the vaccine differs from that of the A2 C4G strain by an amino acid substitution at position 66, numbering from the translation initiation site. The significance of this substitution is unknown.

74. The HN and F genes of hPIV3 and bPIV3 are functionally conserved but share only 35% and 25% of their neutralizing epitopes respectively (Wyke Coelingh et al. 1988) and induce only moderate cross-protective immunity (Clements et al. 1991; Karron et al. 1995b; Karron et al. 1996; Greenberg et al. 2005).

6.3.1 Toxicity or allergenicity of the proteins associated with the introduced genes

75. In the context of the GM virus the introduced genes have been demonstrated to elicit an immune antibody response without the pathology associated with the viruses from which they are derived. Allergic reactions are possible following inoculation with the vaccine.

76. Altered clinical reactivity to subsequent RSV infection was observed in trials of an inactivated RSV vaccine in the late 1960s (Kapikian et al. 1969). This whole killed virus vaccine was administered by injection, resulting in an abnormal immune response and in increased disease symptoms upon subsequent natural exposure to RSV. Modification of the glycoproteins by formalin inactivation has been implicated as a likely cause. Live attenuated RSV candidate vaccines and the GM vaccine have not demonstrated such an abnormal immune response (Tang et al. 2008).

77. In animal and human trials of the GM vaccine no toxicity has been observed (Tang et al. 2008).

6.4 The regulatory sequences

78. Promoters are nucleotide sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Substitution of the hPIV3 HN and F genes also involves substitution of non-coding regulatory sequences between these two genes, however the regulatory sequences of bPIV3 and hPIV3 are similar in nucleotide sequence and are functionally homologous (Haller et al. 2000). Insertion of the RSV F gene involves insertion of some non-coding transcribed sequence flanking the coding sequence, but this does not include the functional sequence motifs, which remain bPIV3 in origin.

6.5 Method of genetic modification

79. To introduce the genetic modifications into the bPIV genome and to recover recombinant virus a reverse genetic system has been developed (Haller et al. 2000). This involved reverse transcription of the viral RNA genome into a cDNA antigenome, and the cloning of this cDNA into a plasmid, allowing the insertion and substitution of DNA sequences using standard subcloning techniques.

80. To rescue recombinant virus, the plasmid containing the antigenomic cDNA, along with plasmids expressing the NC, P and L proteins essential for replication of the RNA genome, were transfected into mammalian cell lines. This resulted in the replication of the viral genome, translation of the viral proteins and assembly into infectious virus particles.

6.6 Characterisation of the GMOs

6.6.1 Stability and molecular characterisation

81. The genomic RNA of the GM virus has been sequenced. After 10 passages in tissue culture cells the recovered virus stably maintained the inserted genes and protein expression (Tang et al. 2003).

82. One of the aims of the proposed clinical trial is to investigate the molecular stability of any GM virus shed by trial participants.

6.6.2 Characterisation of the phenotype of the GM vaccine

83. The phenotype of the GM vaccine has been characterised in model animals such as rats, hamsters, and monkeys (Pennathur et al. 2003; Tang et al. 2003; Tang et al. 2004) and in human clinical trials (Tang et al. 2008). These studies demonstrate that the respiratory tissue tropism is unchanged by the modifications, and also the absence of toxicity in mammalian hosts.

Section 7 The receiving environment

84. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the geographic regions where the release would occur and any relevant properties of these locations; the intended clinical practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release.

85. The proposed dealings involve inoculating infants and young children at clinical facilities listed in Table 1. The handling of the GM vaccine and inoculation of trial participants would be performed in accordance with the *Note for Guidance on Good Clinical Practice* (CPMP/ICH/135/95), and this is expected to ensure safe receipt, storage, handling, dispensing and disposal.

86. As previous clinical trials with the unmodified bPIV3 vaccine have demonstrated that virus was shed in nasal secretions by seronegative infants for approximately 11 days post inoculation (Karron et al. 1995b), the receiving environment would therefore include the homes where the trial participants reside following inoculation as well as any places such as child care facilities that they attend during the period where the virus continues to replicate and be shed.

7.1 Relevant environmental factors

87. Environmental factors relevant to the potential persistence or spread of the GM virus are the presence of susceptible host organisms and any physical conditions that may aid or restrict transmission to these hosts.

88. Adults and children greater than 6 months of age are expected to have been exposed to both hPIV3 and RSV and possess acquired immunity that would limit susceptibility to the GM virus. However, seronegative infants or immuno-compromised adults may be present in the environment where the GM virus may be shed and are likely to be susceptible to infection with the GM virus if exposed to a sufficiently large inoculating dose.

89. Animals that are able or may be able to be infected with bPIV3, such as rodents, may be present in the environment where the GM virus may be shed.

90. Physical conditions such as surfaces that prolong the survival of virus outside of the host may assist the transmission of the GM virus between trial participants and other susceptible hosts. For clinical facilities the applicant states that the World Health Organization Standard Precautions in Health Care (World Health Organisation 2007) would be followed, in addition to clinical practices listed below, to ensure hygiene and control any risks to people undertaking the dealing.

91. For locations outside of the clinical facilities the physical environmental factors influencing the possibility of transmission cannot be fully characterised. However, the presence of susceptible hosts in the environment would be controlled by the exclusion of potential trial participants that may come into contact with immuno-compromised people.

7.2 Presence of related viruses in the receiving environment

92. *Human parainfluenza virus 3* and RSV infections are common in infants in Australia and trial participants may be expected to be exposed to these viruses during the course of the clinical trial.

93. *Bovine parainfluenza virus 3* is common in Australian cattle, but is unlikely to be present within the immediate environment of the 2-24 month old trial participants.

7.3 Presence of the introduced genes, similar genes and encoded proteins in the environment

94. The introduced genes are isolated from organisms that are widespread and prevalent in the Australian environment. Therefore the encoded proteins are present in the Australian environment and people over the age of two years are expected to have come into contact with the organisms and their proteins, and have developed an immune response limiting the likelihood of reinfection.

Section 8 Australian and international approvals

8.1 Australian approvals of GM Bovine parainfluenza virus vaccines

8.1.1 Previous releases approved by Genetic Manipulation Advisory Committee or the Regulator

95. There have been no previous releases of GM *bovine parainfluenza virus* approved within Australia.

8.1.2 Approvals by other Australian government agencies

96. The Regulator is responsible for assessing risks to the health and safety of people and the environment posed by or as a result of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Therapeutic Goods Administration (TGA), Australian Quarantine and Inspection Service (AQIS), Food Standards Australia New Zealand (FSANZ), and Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 3.

97. TGA is the agency with responsibility for approving the experimental use of therapeutic products that are not entered in the Australian Register of Therapeutic Goods, under the Clinical Trial Exemption (CTX) scheme. TGA has approved this trial.

8.1.3 Other Australian approvals

98. Ethical approval is required prior to the commencement of research involving human subjects. Location-specific HREC approval would be required prior to commencement of the trial at any of the clinical sites.

8.2 International approvals of GM bovine parainfluenza virus vaccines

99. Trials of the GM vaccine began in the USA in 2008 and trial participants continue to be actively recruited in that country. Trials will also occur in North and South America, Europe, South Africa and the United Kingdom. The approving agency and any conditions imposed on the trial are detailed in Table 4 below.

Table 4 Overseas applications and approval of trials of the GM vaccine.

Country	Agency	Approval and Conditions
USA	Food and Drug Administration	Investigational New Drug approval
UK	Medicines and Healthcare Products Regulatory Agency	Approval granted
	Department for Environment, Food and Rural Affairs	Approval with conditions for reporting and risk management
Spain	Ministry of the Environment	Approval with conditions for management and control of the vaccine, notification of incidents and submission of a final report
	Agency of Medicinal Products and Medical Devices	Approval with conditions to provide requested information during the course of the trial
Finland	Gene Technology Board	Approval with conditions requiring the reporting of results with potential risks to humans, animals or the environment, and submission of a final report
	National Agency for Medicines	Approval granted
Germany	Paul Ehrlich Institute	Approval granted
South Africa	National Department of Agriculture	Approval for import of GMOs
	Medicines Control Council	Approved with conditions requiring reporting of adverse events, submission of protocol changes for approval, submission of progress reports, adherence to informed consent guidelines and notification of study discontinuation.

Country	Agency	Approval and Conditions
Brazil	National Health Surveillance Agency	Approval granted
	CTNBio (National Biosafety Commission)	Approval granted
Canada	Biologics and Genetic Therapies Directorate, Health Canada	Approval granted

Chapter 2 Risk assessment

Section 1 Introduction

100. 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 5). Risks are identified within the context established for the risk assessment (see Chapter 1), and takes into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

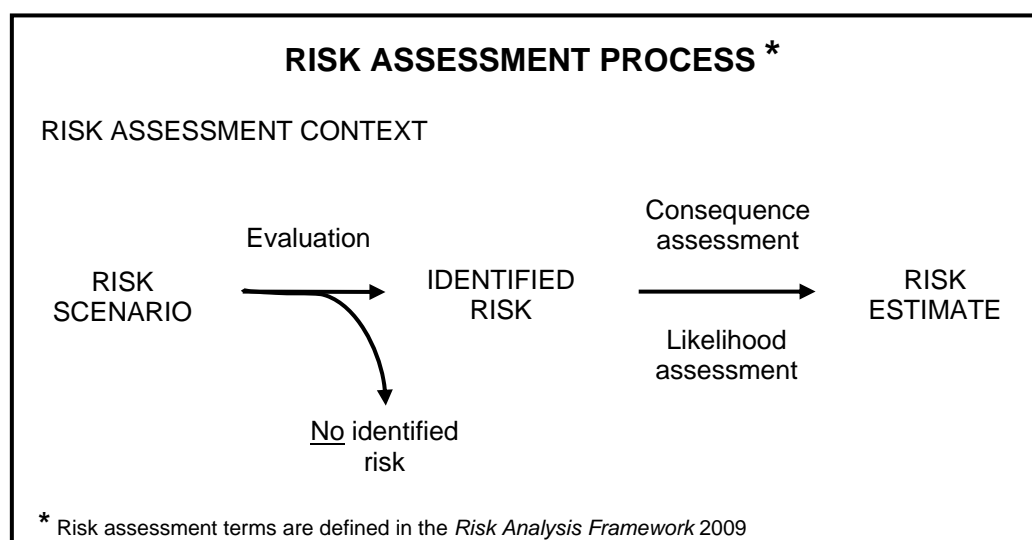


Figure 5 The risk assessment process.

101. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios).

102. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified when a risk scenario is considered to have some reasonable chance of causing harm. Circumstances that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

103. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

104. Identified risks are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk identification

105. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the proposed limits
- the proposed controls
- characteristics of the parent organism
- 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) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- clinical management practices for the GMOs.

106. Five risk scenarios were identified and evaluated in detail later in this section. They are summarised in Table 5 where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment.

Table 5 Summary of risk scenarios from dealings with the GM virus.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to the GM virus material containing proteins encoded by the introduced genes	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • The encoded proteins and their end products occur naturally in the environment and are unlikely to be toxic or allergenic to people or toxic to other organisms • The limited scale, and other proposed limits and controls, further reduce exposure of people and other organisms to products of the introduced genes
Section 2.2 Increased disease burden	2. Exposure of people or animals to the GM virus resulting in infection	Increased virulence/severity of symptoms	No	<ul style="list-style-type: none"> • The limited number of trial participants, and other proposed limits and controls, are expected to restrict exposure to the GM virus • The genetic modifications are unlikely to increase the virulence of the GM virus
Section 2.3 Unintended changes in viral characteristics	3. Changes to the structure and function of the GM virus.	Increased virulence/severity of symptoms	No	<ul style="list-style-type: none"> • Unintended adverse effects, if any, would be minimised by the proposed limits and controls

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.4 Horizontal transfer of genes or genetic elements to other organisms	4. Presence of the introduced genes in other organisms as a result of gene transfer	Increased virulence/severity of symptoms	No	<ul style="list-style-type: none"> The introduced genes are already present in the environment and are available for transfer via natural mechanisms Risk scenario 1 and 2 associated with expression of the introduced genes did not constitute identified risks for people or the environment
Section 2.5 Unauthorised activities	5. Use of the GMOs outside the proposed licence conditions	Potential adverse outcomes identified in Sections 2.1 to 2.4	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

107. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

108. Allergenicity is the potential of a protein to elicit an adverse immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

109. A range of organisms may be exposed directly or indirectly to the introduced genes from hPIV3 and RSV. Trial participants would be intentionally exposed to the GM virus. Clinical staff administering the vaccine or other staff handling the vaccine during transport may be exposed through a spill. People and other organisms may be exposed to the GM virus shed by trial participants in their homes or at other places attended by trial participants for a number of days following the inoculations.

Risk scenario 1. Exposure to GM vaccine material containing proteins encoded by the introduced genes

110. There is a possibility that exposure of people or other organisms to the proteins encoded by the introduced genes could cause a toxic or allergenic response.

111. Expression of the introduced genes would be unlikely to result in the production of novel toxic or allergenic compounds in the GM vaccine. The genome of the GM virus including the introduced genes has been fully sequenced, and none of the viral proteins would be different from viral proteins that occur in the parent virus or the two other viruses that are the source of the introduced genes.

112. People and animals are exposed to the proteins produced by the introduced genes through regular outbreaks of RSV and hPIV3 in the community. No information was identified to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or other organisms (Chapter 1, Section 6.3.1).

113. The proposed limits and controls of the trial (Chapter 1, Section 4.2) would minimise the likelihood of exposure of people and other organisms to the GM vaccine. Human contact

with GM vaccine prior to and during inoculation would be limited to trained and authorised staff. The proposed trial sites are located within hospitals so access to the general public would be minimised. Human contact with GM virus shed by trial participants in the days following inoculation would be limited to people in immediate contact with the trial participants. Exposure of animals to the GM virus would also be limited.

114. **Conclusion:** The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM vaccine containing proteins encoded by the introduced genes is **not an identified risk** and will not be assessed further.

2.2 Increased disease burden from the GM virus

115. Baseline information on the characteristics of, and the factors limiting transmission of bPIV3 is given in Chapter 1. In summary, the virus is known to occur commonly in Australian cattle herds without causing significant disease. Parainfluenza viruses in general require close contact between hosts for transmission, typically via large droplets of nasal secretions making contact with the nasal epithelia of a susceptible host. They are inactivated by exposure to the environment in 10 hours on a damp surface, 2-4 hours on a dry surface or one hour on human skin.

116. Pathways that could lead to an increased disease burden from increased virulence due to the GM virus include transmission of the GM virus to susceptible people or other organisms, expression of the introduced genes conferring lower infectious dose, increased shedding, increased survival time outside of the host cell, or increasing the number of susceptible host organisms, compared to the parent bPIV3 virus.

Risk scenario 2. Exposure of people or animals to GM virus resulting in disease

117. If the GM virus was to persist in the environment through sustained transmission from infected to susceptible hosts, it could increase the exposure of humans and other organisms to the GM virus. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with the GM virus has been considered in Risk scenario 1 and was not considered an identified risk.

118. The level of shedding of the GM virus from trial participants is an important factor in determining exposure to and transmission of the GM virus. Reduced shedding is also an indicator of attenuation.

119. The addition of the hPIV3 HN and F proteins and the RSV F protein could increase the virulence of the GM virus. The hPIV3 HN and F proteins that are incorporated into the GM viral particle are homologous to those of bPIV3 that they replace. One effect of this substitution is to reduce the number of genetic differences between hPIV3 and bPIV3 that attenuate bPIV3 in humans. Preclinical studies suggest that substitution of the hPIV3 HN and F genes for the bPIV3 HN and F genes reduced, but did not eliminate the attenuation of the chimeric bPIV3 compared with hPIV3 in non-human primates (Skiadopoulos et al. 2003; Pennathur et al. 2003). The additional insertion of the RSV F gene could be expected to contribute to attenuation of the GM virus, although in a Syrian Golden Hamster animal model such attenuation was not observed (Tang et al. 2003). As clinical studies have shown an acceptable safety profile with no medically significant vaccine-related adverse events for the GM virus it is highly unlikely that the genetic modifications would increase the virulence of the GM virus.

120. Adults have typically been exposed to both RSV and hPIV3 and have a degree of acquired immunity to these viruses that prevents infection with the GM virus. Of twenty adults inoculated with the GM vaccine at the highest dose (10^6 TCID₅₀) one shed viral

particles. Therefore for infection of an adult to occur following contact with the GM virus in the event of a spill, or with viral particles shed by trial participants in nasal secretions, a similar dose would need to be delivered directly to the nasal epithelia (Tang et al. 2008). This is highly unlikely.

121. Seronegative infants inoculated with the parent bPIV3 virus shed viral particles, but did not transmit the virus to seronegative infants in close contact for 4-6 hours a day over 10 days at a day care centre. Two aims of the proposed study are to determine the minimum infectious dose and the rate of shedding of the GM vaccine in seronegative infants.

122. Viral particles shed in nasal secretions onto surfaces or absorbent materials such as cloth or paper are inactivated over the course of a number of hours. Increasing the length of time that viral particles remain infectious outside the host may increase the number of infectious viral particles that a person or animal may come into contact with through contact with contaminated surfaces or materials. The impact of the genetic modifications on survival of the GM virus is uncharacterised under field conditions, however, the survival properties of human and bovine parainfluenza viruses and of parainfluenza viruses in general are very similar, and substitution of the HN and F genes is unlikely to alter survival outside of the host cell. The phenotypes of the parainfluenza viruses are summarised in Chapter 1, Section 5.3.

123. The host range of the GM virus is not expected to differ from the host ranges of hPIV3 and bPIV3. Both of these viruses replicate in a range of mammalian hosts, and rodents including rats and hamsters are used as small animal models of infection and disease. It is not likely that the host range of the GM virus would differ from the parent viruses. The ability to infect animals that may be present in the homes of trial participants, such as dogs and cats is unknown, however if they are susceptible to infection it is likely that such animals would already have been exposed to hPIV3 and RSV and would possess a degree of acquired immunity to the GM virus.

124. The proposed limits and controls of the trial (Chapter 1, Section 4.2) would minimise the likelihood of transmission of the GM virus proposed for release. The release would be of limited size and at a limited number of locations. The applicant proposes a number of control measures, including exclusion of potential trial participants that are expected to be in contact with infants less than six months of age and likely not to have been exposed to hPIV3, or expected to be in contact with pregnant or immuno-compromised people who may experience a diminished immune response against the GM virus despite previous exposure to hPIV3. The primary factor limiting transmission of GM virus shed from trial participants would be the widespread acquired immunity among people likely to be exposed. With these limits and controls the potential for transmission of the GM vaccine to more susceptible hosts is greatly diminished.

125. **Conclusion:** The potential of the GM virus to increase disease burden due transmission of the virus to susceptible hosts, expression of the introduced genes increasing the infectivity or the number of susceptible host species, or by improving the survival of the GM virus outside of the host is **not an identified risk** and will not be assessed further.

Uncertainty

126. This clinical trial would gather data on the shedding of GM virus from infants that have not previously been exposed to hPIV3. The quantity of viral particles shed following inoculation of seronegative infants is unknown. Wild type bPIV3 has not been transmitted from trial participants to other infants, but it is not known whether the genetic modifications would alter transmission. It has been previously demonstrated that adults are likely to have been exposed to hPIV3 and this previous exposure greatly limits the potential for replication

of the GM virus. However this may not be the case in people who are immuno-compromised. The applicant proposes controls that limit the potential for transmission to immuno-compromised people.

2.3 Unintended changes in viral characteristics

127. Non-segmented negative strand RNA virus genomes code for 5-11 genes and the gene products of individual genes may display pleiotropy⁹, performing more than one function in the process of virus infection and replication (Bukreyev et al. 2006). Gene technology has the potential to cause unintended effects by introducing a gene product that could affect multiple traits. Such pleiotropic effects may include:

- unknown secondary functions conferred by of the introduced genes
- altered expression of other viral genes
- novel traits arising from interactions of the protein encoded by the introduced gene product with viral or host molecules

128. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity, allergenicity, or pathogenicity compared to the parent organism. However, accumulated experience with genetic modification of non-segmented negative strand RNA viruses, including experience with the proposed GM vaccine and parent strains, has not indicated altered toxicity, allergenicity or pathogenicity.

Risk scenario 3. Changes to the characteristics of the GM virus resulting from expression of the introduced genes

129. Although the molecular properties of the GM virus are well characterised, there is some possibility that there could be unexpected changes to the characteristics of the GM virus as a result of the introduced genes.

130. Human and animal trials involving the bPIV3 parent virus, the GM virus with only the hPIV3 HN and F genes, and the GM virus including the hPIV3 HN, F and additional RSV fusion gene, have not demonstrated unexpected changes in the characteristics of the GM virus resulting from the introduced genes. Other considerations relevant to viral characteristics in relation to expression of the introduced genes, have already been discussed in Risk Scenarios 1 to 3, and were not considered identified risks.

131. Insertion of the RSV fusion gene into the non-coding region between the NC and P genes is expected to reduce the transcription of genes downstream of the insertion, reducing the rate of viral growth. This has been confirmed by comparison of viral growth rates in tissue culture (Tang et al. 2003). The difference in growth rates observed in tissue culture was not observed in an animal model, suggesting that factors other than rate of gene transcription limit growth rates *in vivo*.

132. Expression of a modified viral protein or expression in an unusual tissue may create an abnormal immune response. This has occurred in a trial of an inactivated RSV vaccine, where modification of the glycoproteins by formalin treatment resulted in increased symptoms upon subsequent exposure to RSV (Chapter 1, Section 6.3.1). Due to the similarity in the site and process of replication between RSV and bPIV3, the RSV fusion glycoprotein expressed from the GM vaccine is presented to the host immune system in a manner that closely replicates RSV infection and would not create the modifications that led to an abnormal immune response to the formalin inactivated RSV vaccine. No abnormal immune responses to the GM vaccine have been observed in animal and human trials (Tang et al. 2008).

⁹ Pleiotropy is the genetic effect of one gene on apparently unrelated, multiple phenotypic traits (Kahl 2001).

133. **Conclusion:** The potential for an adverse outcome as a result of altered viral structure or function is **not an identified risk** and will not be assessed further.

2.4 Horizontal transfer of genes or genetic elements to other organisms

134. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

135. Baseline information on the presence of the introduced gene or similar genetic elements is provided in Chapter 1, Section 7.3. The introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Risk scenario 4. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

136. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organism and the nature of the introduced genetic material. Risks that might arise from HGT from a GMO to another organism have been recently reviewed (Keese 2008).

137. Horizontal gene transfer by recombination between non-segmented negative strand RNA viruses is extremely rare. It has been reported only once in experiments designed to promote and detect recombination events. The mechanism of recombination involved co-infection of a cell by two strains of the virus, and switching of the RNA polymerase between the different genomes during transcription of the anti-genome template. A degree of homologous pairing between the partial anti-genome template and the alternative genome was required to ensure that transcription recommenced at the appropriate position in the genome to produce a replication competent virus (Spann et al. 2003). Recombination was achieved through the use of high concentrations of viral particles leading to infection of individual cells by many viral particles, and strong selection for recombinants that lacked attenuating mutations present in each parent strain.

138. Recombination between two virus strains infecting the same cell is very rare amongst single negative strand RNA viruses such as bPIV, hPIV or RSV. This has been observed between RSV strains in tissue culture in the laboratory (Spann et al. 2003) but is not expected to occur in nature because of the mechanisms of replication in these viruses.

139. HGT could also result in the presence of the introduced genes in bacteria and in animals or other eukaryotes. However, the introduced sequences were isolated from organisms already widespread in the environment (See Chapter 1, Section 7.3) and already available for transfer via natural mechanisms.

140. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes rather than horizontal gene transfer per se (Keese 2008). If the introduced genes or their end products are not associated with harm to people or other organisms then even in the unlikely event of HGT occurring, they should not pose risks to humans, animals or the environment. Conclusions reached for Risk scenarios 1 – 3 associated with the expression of the introduced genes did not represent

an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

141. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.5 Unauthorised activities

Risk scenario 5. Use of GMOs outside the proposed licence conditions (non-compliance)

142. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to exposure to the GM vaccine outside the scope of the proposed release. The adverse outcomes that this could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

143. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process and assessment of significant risk

144. Five risk scenarios were identified and evaluated. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM virus; or produce unintended changes in viral characteristics. The opportunity for gene transfer to other organisms and its effects if this occurred was also assessed. The possibility of unauthorised activities was also considered.

145. The characterisation of the five risk scenarios in relation to both the seriousness and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The reasons for this include:

- limits on the number of trial participants and locations of the release proposed by PPD
- suitability of controls proposed by PPD to restrict exposure to the GM virus and its genetic material
- widespread presence of the same proteins or sequences encoded by the introduced genes in the environment with no evidence of harm to otherwise healthy people with previous exposure to RSV and hPIV3
- limited ability and opportunity for the GM virus to transfer the introduced genes, that are already widespread in the environment

146. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM vaccine into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment¹⁰.

¹⁰ 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. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Section 4 Uncertainty

147. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

148. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹¹. For clinical trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict exposure to the GMO and its genetic material in the environment, rather than necessarily to treat an identified risk.

For DIR 097 which involves early stage research, uncertainty exists in relation to the characterisation of:

- Risk scenario 2, the likelihood that the introduced genes increase virus shedding in seronegative children in comparison with seropositive children and adults, resulting in exposure of susceptible people or other organisms leading to disease.

149. Additional data, including information to address this uncertainty, may be required to assess possible future applications for the commercial release of the GM virus.

150. Chapter 3, Section 5 discusses information that may be required for future releases.

¹¹ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2009) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

Chapter 3 Risk management plan

151. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. Risk management includes the preparation of a risk management plan to evaluate and treat identified risks, apply general risk management measures, and propose licence conditions. The risk management plan is used to inform the decision-making process. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

152. 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. All licences are required to be subject to three conditions prescribed in the Act.

153. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, 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.

154. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMOs for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

155. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹².

156. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. The *Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

157. The clinical trial of the vaccine has been approved by the Therapeutic Goods Administration under the Clinical Trial Exemption scheme.

158. HREC assessment and approval is an integral part of the governance structure for clinical trials and is also required before the trial can commence.

¹² More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework (OGTR 2009)* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>

Section 3 Risk treatment measures for identified risks

159. Five risk scenarios listed in Chapter 2 were considered in the context of the scale of the proposed release (70 trial participants across six clinical sites over a period of 29 months), the containment measures (Chapter 1, Section 4.2), and the receiving environment (Chapter 1, Section 7).

160. The risk assessment of the risk scenarios concluded that the risks to people and the environment from the proposed trial of GM vaccine are **negligible**. The *Risk Analysis Framework* (OGTR 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

Section 4 General risk management

161. Licence conditions are imposed to control exposure to the GMO and its genetic material in the environment and limit the release to the size and locations requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence and summarised in Chapter 3, Section 4.1.3.

4.1 Licence conditions

4.1.1 Consideration of limits and controls proposed by PPD Pty. Ltd.

162. Chapter 1, Section 4.2 provides details of the limits and controls proposed by PPD in their application, which are discussed in the risk scenarios characterised for the release in Chapter 2. The appropriateness of these limits is considered further below.

163. The permitted dealings are confined to a maximum of 70 trial participants over six clinical sites across six LGAs in the ACT, NSW, Queensland, South Australia, Victoria and Western Australia. The initial proposal was for 35 of the 70 trial participants to be inoculated with the GMO, with the remainder to receive a placebo. However, during the assessment process the applicant requested this be amended to enable the GMO/placebo randomisation to be conducted across all trial participants globally instead of on a national basis.

164. The amended trial protocol now requests that up to 70 trial participants be inoculated with the GMO, with the remainder (if any) receiving the placebo. Increasing the maximum number of individuals potentially inoculated with the GMO by 35 does not significantly alter the potential for non-trial participants or animals to be exposed to the GMO and does not alter the findings of the risk assessment.

165. The applicant has proposed that the trial will be completed by March 2012 for this limited and controlled release

166. These measures proposed by the applicant would limit the exposure of people and animals to the GM virus and have been included as licence requirements.

167. Exclusion of participants from the trial that may come into contact with individuals at risk of disease from exposure to the GM virus will reduce the opportunity for transmission of the GM virus. These include people with decreased antiviral immune function due to pregnancy, chemotherapy, or who are likely to be seronegative such as children under 6 months of age. These exclusion criteria have been included as licence requirements.

168. Inoculations will be performed by trained nurses and/or physicians at clinical facilities in accordance with the World Health Organisation Standard Precautions in Health Care

(World Health Organisation 2007) and the International Conference on Harmonisation Good Clinical Practice Guidelines (ICH 1996). The WHO standard precautions detail appropriate hygiene, personal protective equipment and decontamination procedures to prevent direct contact with the GM virus. These practices and procedures will minimise exposure of people handling the GM virus as part of the trial to the GM virus and have been included as licence requirements.

169. The applicant has proposed standard infection control practices and procedures that minimise exposure to the GM virus. Storage and transport, including any waste or samples containing GM virus, will be required in accordance with relevant regulations. These practices and procedures will minimise exposure of other people and the environment to the GM virus and have been included as licence requirements.

170. The applicant has stated that all waste will be disposed of in accordance with standard clinical waste disposal practices. An audit of waste disposal practices in certified facilities that included clinical facilities has been conducted by the Compliance Investigation Unit of OGTR. An acceptable level of compliance with designated practices was found. These practices and procedures will minimise exposure of other people to the GM virus and have been included as licence requirements.

4.1.2 Summary of measures imposed by the Regulator to limit and control the proposed release

171. A number of licence conditions have been imposed to limit and control the permitted dealings, which are described in detail in the licence. These include requirements to:

- limit the release to a maximum of 70 trial participants inoculated with the GM virus and six clinical facilities
- restrict exposure of at-risk individuals by specific exclusion criteria
- ensure that inoculations be performed by trained nurses and/or physicians at clinical facilities in accordance with standard universal precautions and ICH-GCP¹³
- store and transport all GM virus, including any waste or samples containing GM vaccine, in accordance with relevant regulations¹⁴
- dispose of all waste in accordance with standard clinical waste disposal practices.

4.1.3 Measures to control other activities associated with the trial

172. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs; Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMO for the purposes of, or in the course of, the authorised dealings.

173. Conditions applying to the collection of samples for experimental analyses are also included in the licence conditions.

4.2 Other risk management considerations

174. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

¹³ The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, guidelines for good clinical practice (ICH 1996)

¹⁴ OGTR *Guidelines for the Transport of Genetically Modified Organisms*, IATA Transportation Regulations

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

175. 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 (both individuals and the body corporate)
- 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 applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

176. Before making the decision whether or not to issue a licence for this application (DIR 097), the Regulator has considered the suitability of PPD Pty. Ltd. to hold a licence.

177. The issued licence includes conditions requiring the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

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

4.2.2 Contingency plans

179. The issued licence contains conditions requiring PPD Pty. Ltd. to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan must detail measures to be undertaken in the event of any unintended presence of the GM vaccine outside of the permitted areas.

180. PPD Pty. Ltd. are also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

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

181. The issued licence requires that persons covered by the licence 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.

4.2.4 Reporting structures

182. The licence obliges the licence holder to immediately report any of the following to the Regulator:

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

183. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

4.2.5 Monitoring for Compliance

184. 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.

185. 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.

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

Section 5 Issues to be addressed for future releases

187. Additional information has been identified that may be required to assess an application for a large scale or commercial release of the GM virus, or to justify a reduction in containment conditions. This would include additional data on:

- the potential shedding of GM virus from seronegative trial subjects;
- data on the GMO's capacity to infect or cause disease in animals; and
- data on the genetic stability of the GMO.

Section 6 Conclusions of the RARMP

188. The risk assessment concludes that this proposed limited and controlled release of GM virus to take place in hospitals in ACT, NSW, QLD, SA, VIC and WA, involving a maximum of 70 trial participants and expected to run until March 2012, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

189. The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. If a licence were to be issued, conditions are proposed to restrict the proposed release to the size, locations and duration requested by the applicant as these were important considerations in establishing the context for assessing the risks.

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Appendix A Definitions of terms in the *Risk Analysis Framework* used by the Regulator

Consequence

adverse outcome or impact of an activity

Marginal: minimal adverse health effects; minimal or no damage to the environment or disruption to biological communities

Minor: adverse health effects that are reversible; damage to the environment or disruption to biological communities that is reversible and limited in time and space or numbers affected

Intermediate: adverse health effects that are irreversible; damage to the environment or disruption to biological communities that is widespread but reversible or of limited severity

Major: adverse health effects that are severe, widespread and irreversible; extensive damage to the environment or extensive biological and physical disruption of whole ecosystems, communities or an entire species that persists over time or is not readily reversible

Harm

adverse outcome or impact

Likelihood

chance

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Risk

chance of harm from an activity

Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation

Low: risk is minimal but may invoke actions for mitigation beyond normal practices

Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective

High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis

overall process of risk assessment, risk management and risk communication

Risk analysis framework

guidance on the systematic application of legislation, policies, procedures and practices to risk analysis

Risk assessment

overall process of hazard identification and risk characterisation

Risk characterisation

overall process of consequence and likelihood assessments for an identified risk, and risk estimation

Risk communication

culture, processes and structures to communicate and consult with stakeholders regarding risks

Risk context

parameters within which risk is assessed, managed and communicated

Risk criteria

terms of reference against which the significance of risk is evaluated

Risk estimate

level of risk determined by a combination of consequence and likelihood assessments

Risk evaluation

process of determining if risk requires risk treatment

Risk identification

process of postulating risk scenarios and determining those that warrant detailed risk characterisation

Risk management

mechanisms to control and mitigate risk

Risk management plan

scheme for managing risk posed by dealings with a GMO

Risk scenario

occurrence of a particular set of circumstances that may result in harm from an activity

Risk treatment

process of selection and implementation of measures to reduce risk

Stakeholders

those people and organisations that may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States

includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty

imperfect ability to assign a character state to an entity or activity; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹⁵ on the consultation RARMP for DIR 097

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

Summary of issue raised	Comments
The potential for the GMO to exhibit increased levels of shedding in humans, relative to the parent, should be considered when finalising the risk assessment.	The potential for increased shedding of the GMO from trial participants, and the role this plays in risk scenario 1 and risk scenario 2 is discussed in the RARMP in the context of the proposed limits and controls, which would effectively restrict potential for exposure to the GM virus. The level of shedding of the GMO from seronegative individuals is unknown, and one of the aims of this clinical trial is to gather such information.
Additional data may be required in the event of an application for a wider release of the GMO	The RARMP identifies that there is some uncertainty surrounding the potential level of shedding of GMO from trial participants, and the need to address this prior to any expanded release of the GMO. The final RARMP also identifies that information regarding the potential of the GMO to infect animals, and on the stability of the GMO may be required for the assessment of a wider release application.

¹⁵ GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment, Heritage & the Arts.

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 097

The Regulator received two submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised issues relating to human health and safety and the environment. These 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.

Position (general tone): n = neutral; x = do not support; y = support

Issues raised: C: controls; EN: Environmental issues; H: human health; L: Legal/regulatory OSA: Outside scope of assessment.

Other abbreviations: BPIV: *Bovine parainfluenza virus*; CCI: Confidential Commercial Information; Ch: Chapter; CTX: Clinical trial exemption; GMO: Genetically Modified Organism; HPIV: *Human parainfluenza virus*; HREC: Human Research Ethics Committee; RARMP: Risk Assessment and Risk Management Plan; RNA: Ribonucleic acid; RSV: *Respiratory syncytial virus*; TGA: Therapeutic Goods Administration

Type: I: individual

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	EN, H	Concerned about the potential for gene transfer from the GMO to other RNA viruses, such as <i>Influenza virus</i>	Risks associated with horizontal gene transfer were considered in Risk Scenario 4 (section 2.4). Non-segmented negative stranded RNA viruses, such as <i>Parainfluenza virus</i> have been found to recombine only under artificial conditions. Recombination between other RNA viruses and the GMO in natural situations is considered highly improbable. Furthermore, all the introduced genes in the GMO are already present in the environment and these genes and their products are not associated with harm to people or the environment.
			H	Concerned about the potential for immuno-suppression in trial participants as a result of proteins expressed by the GMO suppressing interferon	Many viruses are able to interact with and suppress the immune system response, including HPIV and BPIV. However, numerous clinical trials have been conducted whereby infants and children have been inoculated with unmodified strains of BPIV, HPIV or the GMO. These trials have found that these strains of BPIV, HPIV and the GMO have acceptable safety profiles.

			H	Although the data suggest the GMO is attenuated by a factor of two of three logs, given the number of virus particles to be administered the data should be looked at more closely.	TGA is responsible for human safety assessment of the participants in clinical trials. The clinical trial of the GM vaccine has been approved by TGA under the CTX scheme. The risks to non-trial participants from inadvertant exposure to the GMO were considered in Risk Scenarios 1 and 2 (sections 2.1 and 2.2). Animal trials and preliminary human trials have indicated that the GMO has an acceptable safety profile. In addition the controls and limits imposed on the trial will reduce the potential for people to be inadvertently exposed to the GMO.
			H	Concerned about the potential for inoculation with the GMO to stop normal mucus secretions rendering the trial participant more susceptible to influenza infection.	There is no direct evidence to suggest that the GMO may affect mucous secretions in trial participants. The F protein from a specific RSV strain (line 19) has been found to cause excess mucus production in some individuals. The RSV F protein present in the GMO does not originate from this source, it is derived from RSV A2 C4G. There is no evidence to indicate that the RSV A2 C4G F protein influences mucus production. The GMO has been tested in clinical trials and demonstrated an acceptable safety profile. TGA is responsible for human safety assessment of the participants in clinical trials. The clinical trial of the GM vaccine has been approved by TGA under the CTX scheme.
			H	Concerned about the interaction of GMO and human immune system, methodology and results of preliminary clinical trials with the GMO that have been declared CCI.	Unpublished results from preliminary and ongoing clinical trials of the GMO have been declared as CCI under section 185 of the <i>Gene Technology Act 2000</i> . CCI material was assessed in considering risks to people and the environment outlined in this RARMP and was made available to prescribed agencies and experts who did not raise any concerns.
2	I	x	L	Is the trial commencement to be halted until the OGTR and HREC have given approval or have the GTR and HREC tacitly given their go-ahead prior to assessment and approval?	The clinical trial can not commence until approval from the TGA, OGTR and a local HREC have been granted.

			H	People with compromised immune systems (such as a foetus) are at risk from exposure to the GMO. Is the finding of a negligible risk appropriate?	The inadvertant exposure of people, including those with compromised immune systems, to the GMO was considered as a risk scenario 2 (section 2.2), which was not found to be an identified risk (<i>i.e.</i> not a plausible risk). The limits and controls proposed by the applicant, including the proposal to exclude participants who would come into contact with pregnant or immunocompromised people, were determined to effectively restrict the potential for people to be inadvertently exposed to a sufficient dose of the GMO to cause infection.
			H, OSA	In the event of a foetus being damaged by the GMO, who would bear legal costs?	See above. Liability and redress issues are outside the scope of the assessment.
			H	The public are entitled to be informed, in a timely manner, of any unintended consequences arising from the trial. CCI must not over-ride this entitlement, and HREC would surely agree?	The licence requires that any unintended effects are reported to the Regulator. The TGA has responsibility for patient safety. TGA operates on an adverse event reporting scheme that ensures notification of adverse events. In addition clinical trials have internal safety monitoring requirements.