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

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 193

Commercial supply of a genetically modified vaccine against infectious laryngotracheitis virus in chickens

Applicant: Bioproperties Pty Ltd

26 October 2022

This RARMP is open for consultation until 21 December 2022.

Written comments on the risks to human health and safety and the environment posed by this proposed supply of the GM COVID-19 vaccine are invited. You may make your submission

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601

or

via email to: ogtr@health.gov.au.

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

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Summary of the Risk Assessment and Risk Management Plan (Consultation Version) for Licence Application DIR 193

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application (DIR 193) for transport, storage and disposal of a genetically modified (GM) vaccine against infectious laryngotracheitis virus (ILTV), as part of its commercial supply as a vaccine for chickens. These activities are classified as Dealings involving the Intentional Release (DIR) of genetically modified organisms into the Australian environment under the *Gene Technology Act 2000*.

Before the GM vaccine can be used, Bioproperties Pty Ltd must also obtain regulatory approval from the Australian Pesticide and Veterinary Medicines Authority (APVMA). The APVMA administers the *Agricultural and Veterinary Chemicals Code Act 1994* (the Agvet Code) to regulate agricultural and veterinary chemical products, including veterinary vaccines. For commercial products, the normal form of approval is through registration. The APVMA can impose conditions on the use of veterinary products via registrations and permits.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed supply of the GM vaccine poses negligible risks to human health and safety and negligible to low risks to the environment. Licence conditions have been drafted for the proposed supply. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Application number	DIR-193
Applicant	Bioproperties Pty Ltd
Project title	Commercial supply of a genetically modified vaccine against infectious laryngotracheitis virus in chickens[1]
Parent organism	Infectious laryngotracheitis virus (ILTV)
Introduced gene and modified trait	Deletion of gene encoding glycoprotein G, which reduces ability of virus to cause disease
Previous releases	The GM vaccine has been previously approved for field trials to vaccinate broiler chickens against ILTV in selected chicken farms in rural Victoria and New South Wales.
Current approvals	The GM vaccine is currently not approved for commercial supply in any region or country.
Proposed locations	Australia-wide
Primary purpose	Commercial supply of the GM vaccine against ILTV in chickens.

[1] The title for the licence application submitted by Bioproperties Pty Ltd is “Commercial supply of Vaxsafe ILT”.

Risk assessment

The risk assessment concludes that risks to the health and safety of people are negligible and the risks to the environment from the proposed supply of this vaccine are negligible to low. Specific risk treatment measures are included in the licence to manage these low risks.

The risk assessment process considers how the genetic modification and activities conducted with the GM vaccine in the context of transport, storage and disposal might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks were considered.

Credible pathways to potential harm that were considered include; the potential exposure of people to the GMO; the potential exposure of animals to the GMO; and the potential for the GMO to recombine with other similar viruses. The potential for the GMO to be released into the environment and its effects were also considered.

The principal reasons for the conclusion of negligible to low risks associated with transport, storage and disposal of the GMO are:

- The GMO has a limited host range, is attenuated and unlikely to cause disease in chickens or other susceptible bird species;
- Infectious laryngotracheitis virus does not cause disease in humans or other organisms except some susceptible bird species;
- The likelihood of accidental exposure to the GMO by people and the environment would be minimised due to well-established transport, storage and disposal procedures that are regulated by each State and Territory; and local councils;
- The GMO would need to be registered with the APVMA, who would impose conditions on the use, transport, storage and disposal of the vaccine; and
- Complementation and recombination of the GMO with another alpha herpesvirus is possible but since the ILT virus was isolated in Australia, similar genetic material would already be present in the environment.

Risk management

The risk management plan concludes the identified negligible to low risks can be managed to protect the health and safety of people and the environment by imposing risk treatment measures. Licence conditions are proposed to prevent the concurrent administration of vaccine with any other ILTV strains and restrict the vaccination to healthy birds. Additional general conditions were also included to ensure that there is ongoing oversight of the GM vaccine.

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

As the risk of recombination leading to novel ILTV strains was assessed as negligible to low, specific risk treatment measures, such as vaccination of only healthy chickens and no concurrent use of live ILTV vaccines were included in the draft licence to ensure that the risk is managed. In addition, draft licence conditions include post-release review (post-market surveillance) to ensure that there is ongoing oversight of the supply of the GM ILTV vaccine and to allow the collection of information to verify the findings of the RARMP. The draft licence, detailed in Chapter 4 of the consultation RARMP, also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

Table of contents

Abbreviations

ACMF	Australian Chicken Meat Federation
APCAH	Asia Pacific Centre for Animal Health
ACT	Australian Capital Territory
AgVet Code	*Agricultural and Veterinary Chemicals Code Act 1994*
AHA	Animal Health Australia
APVMA	Australian Pesticides and Veterinary Medicines Authority
CEO	chicken embryo origin
DAFF	Department of Agriculture, Fisheries and Forestry
dpv	Days post vaccination
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
EPA	Environment Protection Authority
FSANZ	Food Standards Australia New Zealand
GLP	Good Laboratory Practice
GM	Genetically modified
GMP	Good Manufacturing Practice
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HACCP	Hazard Analysis of Critical Control Points
HGT	Horizontal gene transfer
HVT	*Herpesvirus of turkeys*
IR	internal repeat
kb	Kilobase pair of DNA
LGA	Local government area
ml	Milli litre
NSW	New South Wales
NT	Northern Territory
OGTR	Office of the Gene Technology Regulator
Orf	Open reading frame
PCR	Polymerase chain reaction
PPP	Primary Production and Processing
QLD	Queensland
RARMP	Risk Assessment and Risk Management Plan
RNA	Ribonucleic acid
SA	South Australia
TAS	Tasmania
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001

the Regulator	The Gene Technology Regulator
USA	United States of America
VIC	Victoria
WA	Western Australia

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

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

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

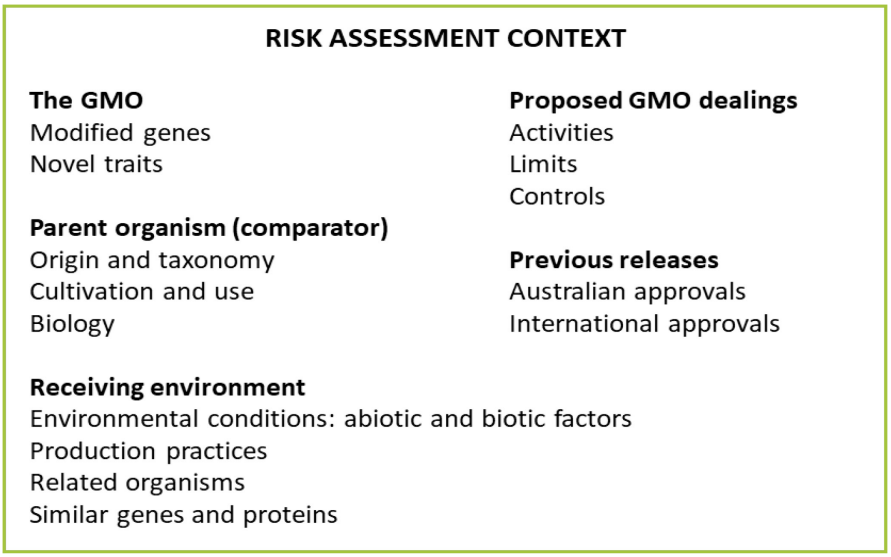

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. Since this application is for commercial purposes, it does not meet the criteria for a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government

authorities and agencies prescribed in the Regulations and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public through a second round of consultation.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA) and the Department of Agriculture, Fisheries and Forestry (DAFF). Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

9. The APVMA provides a national registration and permit scheme for agricultural and veterinary chemical products. It administers the provisions of the *Agricultural and Veterinary Chemicals Code Act 1994* (AgVet Code). For registration, the APVMA assesses whether a new veterinary vaccine meets the criteria set out in the AgVet Code before it is registered in the Register of Agricultural and Veterinary Chemical Products. A new veterinary vaccine that is not registered may be legally used for animal trials, by obtaining a permit from the APVMA.

10. As part of the registration process, the APVMA must first approve the new active constituent; and then assess the quality, safety and efficacy of the vaccine. Quality aspects could include batch-to-batch consistency in vaccine composition, purity and potency. The product must also be manufactured in premises that comply with Good Manufacturing Practice (GMP), which is also audited by the APVMA. Safety aspects include the toxicological profile of the vaccine and its residues, including metabolites and degradation products. The APVMA approves the label, which includes instructions for the handling, storing and directions for supply of veterinary vaccines to ensure safe use. The APVMA would also carry out an environmental risk assessment to minimise environmental risks. The APVMA may also impose conditions on a permit for the supply of veterinary vaccines for research purposes. The States and Territories are responsible for the enforcement of the conditions associated with an APVMA registration. FSANZ develops the food standards in the Food Standards Code with advice from other government agencies and input from stakeholders. The Standards in the Food Standards Code are legislative instruments and cover the composition of some foods, such as dairy, meat and beverages. FSANZ is also responsible for labelling of packaged and unpackaged food, including specific mandatory warnings or advisory labels.

11. Food standards are enforced by the states and territories (usually their health or human services departments) or, in some cases, by local government. These authorities regularly check food products for compliance with the Food Standards Code.

12. FSANZ has developed the Primary Production and Processing (PPP) Standard for Poultry Meat (Standard 4.2.2) (Food Standards Australia New Zealand, 2012) and PPP Standard for eggs and egg products (Standard 4.2.5) (Food Standards Australia New Zealand, 2018). PPP Standards (which only apply in Australia) aim to strengthen food safety and traceability throughout the food supply chain from paddock to plate. The standard introduces new legal safeguards for growing live poultry and requires poultry growers to identify and control food safety hazards associated with poultry farming. Poultry processors are also required to identify and control food safety hazards associated with poultry processing (which includes the slaughtering process) and verify the effectiveness of the control measures.

13. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies would not be re-assessed by the Regulator.

14. For the commercial supply of a GM veterinary vaccine, dealings regulated under the Act include the transport, storage and disposal of GMOs. The Regulator has assessed risks to people as a consequence of conducting these activities and risks from persistence of the GMO in the environment.

Section 2 The proposed dealings

15. Infectious laryngotracheitis virus (ILTV) or *Gallid herpesvirus 1* is a viral respiratory tract infection, which is endemic in Australia and outbreaks commonly occur in Victoria and New South Wales. Once an outbreak occurs, it can have a major impact on the commercial poultry industry. There are currently three non-GM live attenuated ILTV vaccines that are registered for use in Australia (APVMA PubCRIS database). These vaccines consist of attenuated strains of ILTV (SA2, A20 and Serva).

16. Bioproperties Pty Ltd (Bioproperties) is seeking authorisation for the commercial supply of a genetically modified (GM) vaccine (known as Vaxsafe ILT®) to prevent ILT disease in commercial poultry farms Australia-wide. The vaccinated chickens would enter general commerce, including use in human food. Bioproperties is also seeking authorisation to test different modes of administration of the GM vaccine.

17. For the ongoing commercial supply of the GM vaccine and testing of different modes of administration, the dealings assessed by the Regulator are to:

(a) conduct experiments with the GMO;

(b) transport the GMO;

(c) dispose of the GMO;

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

2.1 Details of the proposed dealings

18. The GM vaccine would be manufactured in Australia under GMP conditions in PC2 certified facilities under a Notifiable Low Risk Dealing (NLRD) authorisation. Labelling or packing of vials for storage or transport may occur outside the PC2 facility. Large scale manufacturing of the product (>25L) would require a Dealings Not Involving Intentional Release (DNIR) licence from the OGTR. The product would need to be registered through the APVMA before commercial use. As mentioned in Section 1.1, the APVMA would also approve the labels for the GM vaccine, which would contain instructions for the handling, storing and directions for supply to ensure safe use. Bioproperties are currently conducting trials of this vaccine under an APVMA permit (PER91758) and an OGTR licence (DIR-154).

19. The vaccine would be transported to a distribution centre freeze-dried in glass vials. These vials would be packed into trays, wrapped with plastic cling wrap and placed into Styrofoam boxes filled with dry ice. The Styrofoam boxes would then be placed within cardboard boxes for distribution. The GM vaccine would be dispatched to a variety of commercial poultry farms throughout Australia using couriers. Each individual vial would be labelled with an APVMA approved label indicating the contents of the vial and an accompanying leaflet with instructions for use, storage, and disposal. During transport, the outer most cardboard box would include the name, address, and contact details of the sender, so that the sender can be contacted should the container be lost, damaged or misdirected.

20. The GM vaccine would be administered to chickens in commercial poultry where there is a history or risk of ILTV infection or in the event of an outbreak in the region.

21. The applicant has stated that the GM vaccine would be used as per the currently approved APVMA research permit. Note that the GM vaccine is currently not registered as a commercial product with the APVMA and any commercial GM vaccine must be stored and used as per final registration with the APVMA.

22. Administration of the vaccine via eye drop would be carried out by farm personnel under the direction of a veterinarian. The applicant proposes to register this method of administration with the APVMA. Administration via eye drop and drinking water are currently approved under both APVMA PER91758 and DIR-154. Bioproperties also intend to test other methods of administration to optimise the vaccination efficacy (e.g. *in-ovo* or coarse spray). This RARMP will consider vaccine administration via eye drop, *in ovo,* drinking water or coarse spray.

23. All residual vaccine and associated waste which has come in to contact with the GM vaccine (such as syringes, vials and eye droppers) would be discarded into solutions containing appropriate disinfectant (e.g. bleach) prior to disposal. The disposal of all other waste (e.g. litter and dead carcases) is usually carried out via composting, burial, rendering (high heat processing of poultry by products not intended for human consumption) or disposal in landfill, and would be carried out in accordance with State/Territory, local council and Environmental Protection Agency (EPA) requirements; the poultry industry biosecurity standards (Department of Agriculture Fisheries and Forestry, 2009a, b; Animal Health Australia, 2020; Australian Chicken Meat Federation, 2020); and conditions imposed by the APVMA registration of the GMO. These requirements and guidelines all aim to limit the exposure of other people or animals to the waste by managing populations of pests (e.g. dogs, cats, rodents, wild birds and darkling beetles).

Section 3 Parent organism

24. The GM vaccine is derived from the CSW-1 strain of avian infectious laryngotracheitis virus (ILTV), which was originally isolated from the Glenfield, NSW outbreak in 1959. ILTV is a member of the *Herpesviridae* family, *Alphaherpesviridae* subfamily and is also known as *Gallid herpesvirus 1* (Ou and Giambrone, 2012; Gowthaman et al., 2020). The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with the GM vaccine. As such, the relevant biological properties of ILTV will be discussed here.

25. A characterisation of ILTV isolates in the United States (Oldoni and Garcia, 2007) and Australia (Kirkpatrick et al., 2006; Blacker et al., 2011; Agnew-Crumpton et al., 2016) independently categorised ILTVs into 9 and 10 genetic groups, respectively, according to the differences in restriction enzyme patterns (restriction fragment length polymorphism; RLFP).

3.1 Pathology

26. ILTV is a viral respiratory tract infection which results in severe production losses due to weight loss, decreased egg production and increased mortality of infected chickens. Clinical signs generally appear between 6-12 days following natural exposure and 2-4 days after experimental intratracheal infection (Bagust et al., 2000; Fuchs et al., 2007). The disease can be characterised into mild or severe forms (Ou and Giambrone, 2012; Gowthaman et al., 2020). The clinical course of the diseases varies from 11 days to 6 weeks depending on the form of the disease contracted (Gowthaman et al., 2020). ILTV can also form a latent infection 7-10 days following tracheal exposure (Bagust et al., 2000).

27. Typical clinical signs of the mild form of the disease include decreased egg production, watery eyes, nasal discharge, and conjunctivitis. In severe forms of the disease, signs also include laboured breathing, wheezing, coughing, gasping and expectoration of blood-stained mucus. Birds can die from this disease due to suffocation, as the windpipe becomes completely blocked. The mortality rate varies between 5-70% depending on the disease severity, typically 10-20% mortality in most cases, but it can be as high as 90-100% in severe cases (Bagust et al., 2000; Ou and Giambrone, 2012; Gowthaman et al., 2020).

3.2 Structure and genomic organisation

28. ILTV has a linear double-stranded DNA genome approximately 150-155 kilo base pairs (kb) in length. The genome contains a unique long (UL), unique short (US), and inverted repeats sequences (IR), which flank the US sequence (Figure 2) (Fuchs et al., 2007; Lee et al., 2011; Piccirillo et al., 2016). The

ILTV genome is predicted to have three origins of viral DNA replication, one (ORI_L) located within the UL region, and two ORI_S within the US region, which encodes for various proteins that form the virus (Fuchs et al., 2007).

29. The virus is comprised of four distinct structural elements; the envelope, tegument, nucleocapsid and core (Figure 2). The lipid envelope contains glycoproteins, which are responsible for viral replication and elicit humoral and cell-mediated immune responses in infected host (Gowthaman et al., 2020). Contained within the envelope is the nucleocapsid, which surrounds the core comprising the viral DNA. The region between the envelope and capsid is the tegument, which contains viral proteins likely to have important roles in modulating virus-host interactions (Gowthaman et al., 2020). The virus particle varies in size between 200 and 350 nm, since ILTV incorporates large but variable amounts of tegument proteins (Fuchs et al., 2007).

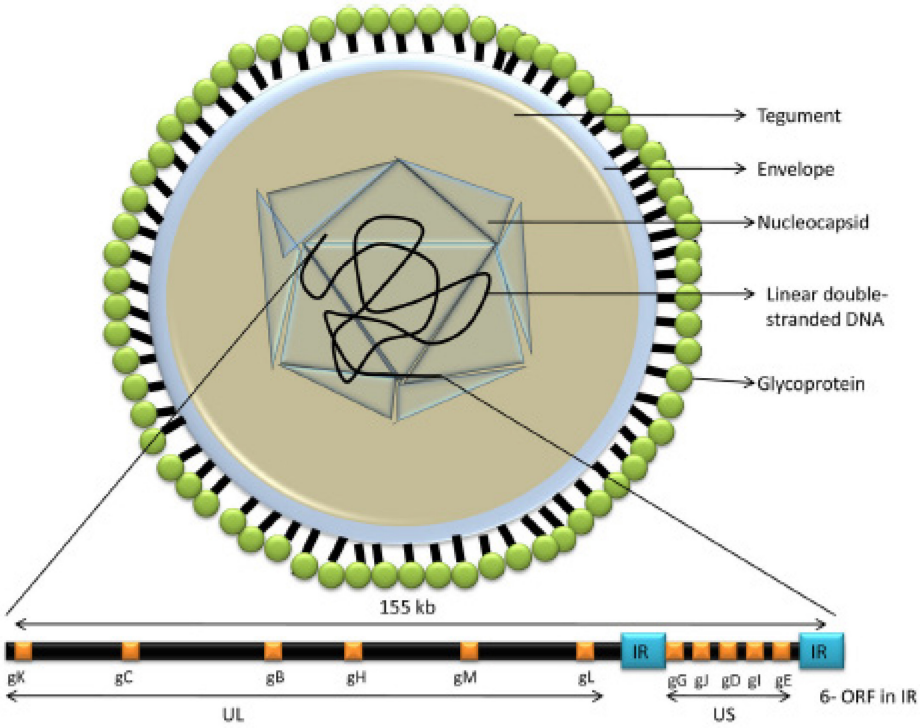

Figure 2. Structure and genome of ILTV. IR: internal inverted repeat; UL: unique long; US: unique short; IR: inverted repeat; and g: glycoprotein. Adapted from Gowthaman et al. (2020).

3.3 Viral infection and replication

30. ILTV replication occurs during the first week of infection. Replication mainly occurs in the epithelial cells in the nasal passage and the eye mucosa, which is the main site of transmission and pathology. Infection is initiated by attachment of virus glycoprotein to the cell membrane receptor followed by fusion of the envelope with the host cell plasma membrane. The tegument and nucleocapsid are transported into the cytoplasm. Once in the cytoplasm, viral DNA is released from the nucleocapsid and migrates into the nucleus through nuclear pores (Fuchs et al., 2007; Gowthaman et al., 2020).

31. Transcription and replication of viral DNA occur within the nucleus in a highly regulated, sequentially ordered cascade like other alpha herpesviruses. The viral DNA is not known to integrate into the host genome. Viral DNA replicates and is packaged into nucleocapsids within the nucleus, before migrating through the nuclear membrane to the cytoplasm, and subsequently associates with the tegument proteins. The nucleocapsids are re-enveloped in the Golgi region and mature particles are then released by exocytosis (Fuchs et al., 2007; Gowthaman et al., 2020).

3.4 Classes and potential for recombination of ILTV

32. As mentioned in Section 3, ILTV strains can be categorised into different classes based on RFLP PCR of ILTV genes (*MspI, HaeIII and Fok1*) within genomic regions (TK, ICP4, ICP18.5 and ORF-BTK). ILTV strains with the same RFLP pattern were placed into one class. In Australia, this method has been used to identify 10 different classes of ILTV (Table 1) (Blacker et al., 2011; Agnew-Crumpton et al., 2016).

Table 1 Classes of ILTV viruses based on their RLFP patterns using restriction enzymes (MspI, HaeIII and Fok1). Adapted from Blacker et al. (2011); Agnew-Crumpton et al. (2016).

Class	RLFP pattern				Vaccine strain equivalent
1	A	A	A	A	A20, SA2
2	B	B	B	B	-
3	B	A	C	B	-
4	B	C	C	B	-
5	A	A	A	B	-
6	B	B	C	B	-
7	B	D	C	B	Serva
8	A	D	C	B	-
9	A	D	A	A	-
10	A	A	C	B	-
Restriction enzyme	*MspI*	*HaeIII*	*HaeIII*	*Fok1*	
PCR product	TK	ICP4	ICP18.5	ORF-BTK	

33. ILTV class 1 consists of the A20 and SA2 vaccine strains; Class 7 corresponds to the Serva vaccine strain; and Classes 2-6 and 8-10 comprise other field strains isolated from outbreaks in commercial flocks in Australia (Blacker et al., 2011; Agnew-Crumpton et al., 2016). The standard laboratory strain in Australia, CSW-1 (Lee et al., 2013) belongs to Class 4 (Asif et al., 2022) and is more genetically related to the Serva than SA2 strain.

34. ILTV classes 8 and 9 are phylogenetically close to class 7 (Serva), indicating a close genetic relationship between the circulating field and vaccine strains (Blacker et al., 2011). Furthermore, the emergence of classes 8 and 9 coincided with the introduction of the Serva vaccine in 2007 (Agnew-Crumpton et al., 2016). Further genome analysis also suggested the possibility that class 8 and 9 ILTVs are the result of recombination between the co-circulating A20, SA2 and Serva vaccine strains (Lee et al.,

2012). Classes 8 and 9 ILTVs were shown to be more virulent than their parent strains when studied *in vivo* in chickens (Lee et al., 2012). Class 9 was shown to have improved growth kinetics and transmission potential over previous dominant field strains (Lee et al., 2015).

35. ILTV class 10 was isolated from samples obtained from commercial poultry farms and a few backyard flocks during Australian disease outbreaks in NSW in 2013. These flocks were vaccinated with one or a combination of the three available ILTV vaccines. Class 10 shares genomic regions with classes 1 (SA2), 7 (Serva), 2 and 8 viruses, suggesting that it may have emerged due to recombination events between these classes of ILTV (Agnew-Crumpton et al., 2016).

36. More recently in Australia, whole genome analysis of a field strain of ILTV identified a new class (class 7b), which is believed to be the result of a recombination between a circulating virus, possibly class 9, which was the predominant strain in Australia at the time, with the Serva vaccine (now reclassified as class 7a) (Sabir et al., 2020).

37. Using the BLAST online tool from the National Center for Biotechnology Information, the nucleotide sequence identity of the whole genome of CSW-1 strain[2] was compared with other ILTV strains. The results showed that CSW-1 has 99.82% identity with the Serva[3], 99.70% identity with the SA2[4] and 99.69% identity with the A20 strain[5].

38. It has also been shown that live attenuated vaccines are capable of reverting to WT strains after bird-to-bird passages so could potentially result in a more virulent ILTV in the field. A study in Canada has shown that vaccine revertant strains are more pathogenic and have a higher transmission rate compared to field strains (Perez-Contreras et al., 2021). In contrast, in a separate study by the same research group, the same revertant strain was shown to be as pathogenic as field strains but replicated better in primary replication sites (e.g. trachea and oral swabs) (Elshafiee et al., 2022). However, interestingly chickens infected with the field strain showed more severe signs of disease than the revertant strain in this study (Elshafiee et al., 2022).

39. Recombination requires a cell to be simultaneously co-infected by two viruses and is more likely to occur at the peak of replication. Vaccination may reduce recombination events when hosts are subsequently infected (Loncoman et al., 2018). Recombination between different ILTV strains has been shown to occur under laboratory conditions (*in vitro*, *in ovo* and *in vivo)* by simultaneous co-infection (Loncoman et al., 2017; Fakhri et al., 2020). Under field conditions, the temporal delay between the infection of the first virus and a second strain of ILTV results in a reduced likelihood of recombination events.

40. Based on the available evidence, recombination between ILTV strains is plausible and may have been facilitated by various conditions under which the ILTV vaccines were used. Possible reasons include the introduction of the European Serva strain into the Australian environment, the use of different ILTV vaccines on a single flock, and possible reactivation of ILTV prior to vaccination. These findings show that the use of multiple attenuated ILTV vaccines under conditions imposing high selective pressures may foster recombination between co-circulating viruses and lead to selection of more virulent or transmissible progeny (Coppo et al., 2013; Agnew-Crumpton et al., 2016; Fakhri et al., 2020).

[2] (Genbank accession number: JX646899.1)
[3] (Genbank accession number: HQ630064)
[4] (Genbank accession number: JN596962.1)
[5] (Genbank accession number: JN596963.1)

3.5 Epidemiology

3.5.1 Host range and transmissibility

41. The chicken is the primary host and reservoir for ILTV. Chickens older than 3 weeks of age are most susceptible to ILTV (Ou and Giambrone, 2012). Natural infection with ILTV has also been observed in pheasants, peafowl (Crawshaw and Boycott, 1982) and turkeys (Portz et al., 2008). Other domestic or feral avian species (e.g. quail, guinea fowl, pigeons, starlings, sparrow, crows and doves) appear to be resistant to disease caused by ILTV (Seddon, 1936). Although, ILTV does not cause disease or mortality in ducks, they can form neutralising antibodies to ILTV when infected experimentally (Yamada et al., 1980). There has not been any reported natural infection of ducks with ILTV.

42. ILTV has not been shown to be transmitted to eggs or on egg shells (Gowthaman et al., 2020) and is not known to infect humans, other non-avian vertebrates or other organisms including invertebrates, plants, microorganisms and aquatic organisms.

43. ILTV is mainly transmitted by direct contact of infected and uninfected hosts through the respiratory, conjunctival and oral routes (Yegoraw et al., 2021). ILTV can be detected via PCR in stool samples (Roy et al., 2015), blood and plasma (Yegoraw et al., 2021). However, a separate study by Yegoraw *et al*, demonstrated no transmission of ILTV via the excreta, blood, or plasma of infected chickens to uninfected chickens (Yegoraw et al., 2020).

44. ILTV can also be indirectly transmitted between farms through contaminated equipment, clothing, trucks and litter (Yegoraw et al., 2021). Dispersion of poultry dusts has been suggested to be a source of ILTV outbreaks between farms as ILTV positive DNA has been previously detected in poultry dust from infected chickens (Yegoraw et al., 2021). Poultry dust, which is mainly composed of feather dander, fine particulates from bedding, feed, and faeces were found to show high levels of ILTV DNA (Roy et al., 2015). However, dust samples obtained from isolator cages containing ILTV positive chickens at 3, 7 and 14 days post-infection, were unable to infect other uninfected chickens either as dust or when diluted in an aqueous solution (Yegoraw et al., 2021) and in a separate study, no viable ILTV was isolated from PCR positive dust samples (Bindari et al., 2020). These data suggest, while ILTV can be detected in dust samples, it is unlikely to cause transmission to uninfected chickens.

45. Windborne transmission has also been implicated in transmission between farms (Johnson, 2005) as high levels of ILTV DNA (SA2 and A20 strains) has been detected in dust and bedding material (Roy et al., 2015). In addition, it has been suggested that low air humidity contributed to higher detection of ILTV DNA in air samples (Brown et al., 2020). Two separate laboratory-based airborne transmission studies showed poor airborne transmission of vaccine strains (A20, SA2 and Serva) compared to virulent field strains (Class 9 and 10) (Yegoraw et al., 2020; Yegoraw et al., 2021). It is thought that this difference may be attributed to the higher replication and shedding rate of the field strains compared to the attenuated vaccine strains. Interestingly, the SA2 vaccine strain, which is known to have residual virulence showed slightly higher capacity for airborne transmission than other vaccine strains (Yegoraw et al., 2020). Together, these data suggest a variability in airborne transmission with different strains of the virus and supports that airborne transmission is possible although less so in vaccine strains.

46. The larvae and adult darkling beetles (*Alphitobius diaperinus*) are prevalent in poultry facilities. ILTV DNA has been detected in the darkling beetles for up to 42 days after an outbreak, implicating it in the transmission of ILTV (Ou, 2012). However, it is unclear if ILTV detected in infected beetles is viable and can cause disease when ingested by birds.

3.5.2 Bio-distribution and shedding

47. The cells lining the nasal cavity, conjunctiva, tracheal mucosa and upper respiratory tract are the major sites of ILTV replication (Coppo et al., 2013; Gowthaman et al., 2020). Experimentally, ILTV is shed 2 days post-infection and 4 days prior to appearance of clinical signs. ILTV can be shed in respiratory secretions for 10 days post-infection (Gowthaman et al., 2020).

48. ILTV can persist for up to 3 months in tracheal exudates at temperatures between 20-23°C, if protected from light (Bagust et al., 2000). However, the decay process of dead chickens will likely shorten ILTV survival. As mentioned in Section 3.5.1, ILTV has also been detected in excreta of chickens via PCR, but it has not been shown to be viable and cause transmission to uninfected chickens (Yegoraw et al., 2020) and no viable ILTV was obtained from cloaca (excretory opening) swabs with chickens infected with ILTV (Oldoni et al., 2009). These data suggests that ILTV is inactivated during gut passage (Yegoraw et al., 2020).

3.5.3 Latency

49. The ability to establish latency to evade the host immune system is the major biological survival mechanism of herpesviruses. Like other herpesviruses, ILTV can establish latent infections within their hosts. The latent infection is characterised by a shutdown of virus replicative functions and the inability to detect infectious virus (Wilson and Mohr, 2012).

50. The trigeminal ganglion, which provides the main sensory innervation to the tissues of the upper respiratory tract, including the trachea, is the main site of latency for ILTV (Williams et al., 1992b; Ou and Giambrone, 2012; Gowthaman et al., 2020). Chickens with latent infections, that have recovered from ILT disease, no longer showed symptoms. In chickens who had recovered from an ILTV infection, ILTV DNA was detected by PCR in the trigeminal ganglion 31-, 46- and 61-days post-infection (Williams et al., 1992b).

51. A study, involving chickens that have recovered from CSW-1 ILTV or SA2 infection, showed reactivation of the virus in 37.5% (6/16) of chickens between 3 to 15 months post-infection, and 44.4% (4/9) between 2 to 10 months post-infection (Bagust, 1986). This study showed that both field and vaccine strains of ILTV can establish long-term latent infections.

52. The reactivation and shedding of ILTV could occur when birds are stressed (e.g. during laying of eggs or transport) (Ou and Giambrone, 2012; Coppo et al., 2013; Gowthaman et al., 2020). This could lead to intermittent shedding and spread of disease to susceptible birds (Williams et al., 1992a).

3.5.4 Prevalence

53. ILTV is considered endemic in Australia and outbreaks have predominantly recurred in chicken farms in Victoria and NSW. Historically, these outbreaks were caused by different classes of ILTV. From 2007 to 2009, ILTV class 2 was responsible for most outbreaks in Victoria, while the majority of outbreaks in NSW were identified as class 8. Based on the samples tested, Class 4 (CSW-1) and 5 were not identified to cause outbreaks in NSW or Victoria during this period (Blacker et al., 2011).

54. However, between 2009 and 2015, class 2 and class 8 were replaced by class 9, which became the predominant strain in Australia. Class 4 (CSW-1) was not detected during this outbreak (Agnew-Crumpton et al., 2016).

3.5.5 Controls and vaccine administration methods

55. Vaccination and biosecurity procedures are the main methods used to control ILTV in the poultry industry because there are no effective treatments available (Gowthaman et al., 2020). Two main types of vaccines are used commercially to control ILTV worldwide (attenuated live vaccines and recombinant viral vectored vaccines).

56. Virulent strains of ILTV were first used in the 1930s to vaccinate birds against ILTV and were considered the first effective vaccine for a major avian viral disease (Coppo et al., 2013). Subsequently, attenuated live viral vaccines were produced by consecutive passages of virulent stains in cell cultures (tissue culture origin; TCO) or in embryonated hen eggs (chicken embryo origin; CEO) (Coppo et al., 2013). CEO and TCO vaccines are still widely used worldwide to control ILTV outbreaks (Menendez et al., 2014) but CEO vaccines confer better protection than TCO vaccines (Gowthaman et al., 2020). The three ILTV vaccines commercially approved for use in Australia (SA2, A20 and Serva) are CEO vaccines (Blacker et al., 2011).

57. Vaccines (CEO and TCO) can contribute to novel new strains through recombination between vaccine strains and remain latent in vaccinated hosts leading to further outbreaks of ILTV. Multiple bird-to-bird passaging of CEO vaccines can also contribute to increased virulence and ILTV vaccines have been implicated in outbreaks in Canada and USA (Blacker et al., 2011; Ou and Giambrone, 2012; Menendez et al., 2014; Barboza-Solis et al., 2021).

58. Due to the limitations of TCO and CEO vaccines, recombinant viral vectored vaccines (e.g. turkey *herpesvirus*; HVT or *fowlpox virus*; FPV) were designed. These recombinant vaccines are modified to express ILTV glycoproteins that can elicit protective immune responses in vaccinated birds, and are now used commercially in some poultry-producing regions around the world (Coppo et al., 2013; Barboza-Solis et al., 2021). These recombinant ILTV vaccines do not cause latent infections and virulent reversions (Ou and Giambrone, 2012). Although these viral vectored vaccines were able to prevent mortality and disease severity, this type of vaccine conferred less protective immunity compared to TCO or CEO vaccines (Coppo et al., 2013).

59. Commercial TCO or CEO vaccines are commonly administered via eye drops, drinking water or coarse spraying. Administration using the eye drop method involves placing droplet(s) of the vaccine into both eyes of the chicken with a special dropper to deliver an accurate volume. Administration by drinking water is done by mixing the vaccines with water in the drinking water troughs or drinker systems. When administered in drinking water, vaccine is provided in an amount of water calculated to be consumed within 3-4 hours, and no additional water is supplied until all the water has been consumed. Administration by coarse spraying is carried out in sheds and usually under veterinary advice. It involves spraying the chickens with vaccine suspension. The eye drop method is considered safer to chickens and more efficient in conferring immunity due to the ability to control the dose administered compared to administration via drinking water or coarse spraying (Ou and Giambrone, 2012; Gowthaman et al., 2020). Coarse spraying may also cause serious reactions in chickens due to excess dosing and aerosol droplets penetrating deeper into the respiratory tract of chickens if the droplets are too small (Ou and Giambrone, 2012). In Australia, eye drops and drinking water administration methods are the only methods currently approved for ILTV vaccines registered with the APVMA (APVMA PubCRIS database). All three registered vaccines in Australia do not have a withholding period and can be administered at any point in time in the life of chicken.

60. Current commercially available recombinant FPV vaccine (in USA) are administered *in ovo* (in eggs) or intramuscularly (Coppo et al., 2013). *In ovo* administration is normally only conducted in commercial hatcheries where the vaccine is injected into fertile eggs at approximately day 18 of incubation, manually or by an automated machine (Grimes, 2018).

3.5.6 Stability and decontamination methods

61. As ILTV is an enveloped virus, it is sensitive to heat, organic solvents (e.g. ether, chloroform, or other lipolytic solvents) and oxidising agents (e.g. bleach) (Gowthaman et al., 2020). Treatment with 3% cresol or a 1% lye solution are known to be able to kill ILTV (Ou and Giambrone, 2012). The fumigation of chicken farms with 5% hydrogen peroxide also completely inactivated ILTV (Ou and Giambrone, 2012). ILTV has also been shown to be inactivated by exposure to ultraviolet light for 60 seconds (Deshmukh and Pomeroy, 1969).

62. The sensitivity of ILTV to heat varies depending on the strain. Very early studies in 1966, that are reviewed in more recent publications, have suggested that ILTV can remain in respiratory excretions and chicken carcasses (10 days to 3 months at 13-23°C), in deep litter (3-20 days at 11-24.5°C), droppings (3 days at 11-19.5°C) and buried carcasses (3 weeks) (Ou and Giambrone, 2012; Gowthaman et al., 2020). Litter containing ILTV heated at 38°C for 24 hours in an oven or composted for 5 days resulted in no detection of ILTV by PCR. Similarly, ILTV was not detected after addition of commercial litter treatment chemicals (e.g. aluminium sulphate (Al+Clear®)) that reduces ammonia and pH in litter (Giambrone et al. 2008).

63. ILTV vaccine DNA has been detected at high levels in dust from laboratory chicken cages at 28 days after inoculation of chickens with either A20 or SA2 vaccine. Litter samples from these laboratory chicken cages also contained ILTV vaccine DNA which was shed from the vaccinated chickens (Roy et al. 2015). However, in the same study mentioned in Section 3.5.1, ILTV in dust samples from A20 and Serva vaccinated chickens was not transmitted to uninfected chickens (Yegoraw et al., 2021). In addition, a separate study also showed that no viable ILTV was isolated from PCR positive dust from poultry farms, while viable ILTV was isolated from the control dust spiked with ILTV (Bindari et al., 2020). It was also observed in the same study that drying and freeze thawing is capable of reducing infectious ILTV (Bindari et al., 2020).

64. Biofilms in drinking water lines have been suspected of being a source of ILTV as it is a common method of administering ILTV vaccine. After running a CEO ILTV vaccine mixed with water into lines and flushing the lines with tap water three times, ILTV vaccine DNA was still detected in water from the lines for up to 21 days. Chickens drinking from this water line tested positive for ILTV DNA up to 21 days after flushing with water. Using the same method above, sanitising solutions were held for 24 hours in the water lines and then flushed with tap water to determine the effectiveness of the sanitising agents. ILTV vaccine was not detected in the water lines after sanitising with sodium bisulfate (0.31 mL/L) or hydrogen peroxide (30 mL/L) solution. However, ILTV vaccine was still detected after treatment with citric acid (3.05 mL/L) or sodium hypochlorite (0.19 mL/L). Chickens tested positive for ILTV DNA after drinking from the water lines treated with citric acid or sodium hypochlorite, while they tested negative after sodium bisulfate or hydrogen peroxide (Ou et al. 2011).

65. After an ILT disease outbreak in California affecting over 50 chicken farms, it was shown that ILTV was no longer isolated from chickens introduced into the farms that employed a thorough decontamination regime. This regime involved heating the farm shed to a minimum of 37°C for 100 hours, thorough cleaning and disinfection of the farm facilities and all equipment, heating again to a minimum of 37°C for 100 hours and a downtime of 21 days where flocks were not introduced into the farm (Chin et al., 2009).

Section 4 The GM vaccine - nature and effect of the genetic modification

4.1 The genetic modifications

66. The wild type parent strain was originally derived from the virulent strain isolated from a field outbreak of ILT in Glenfield, NSW in 1959 (CSW-1 strain). The CSW-1 strain underwent several passages in cell lines before undergoing the genetic modifications described below.

67. The GMO is a live attenuated virus with a deletion of the gene encoding glycoprotein G (gG). The gG gene was removed by a series of targeted homologous recombination steps (Figure 3). Initially the gG gene was replaced with the enhanced green fluorescent protein (eGFP) gene, resulting in ΔgG(eGFP) ILTV. This eGFP gene was then removed from the ΔgG(eGFP) ILTV genome to create the GMO (ΔgG ILTV) (Devlin et al., 2006).

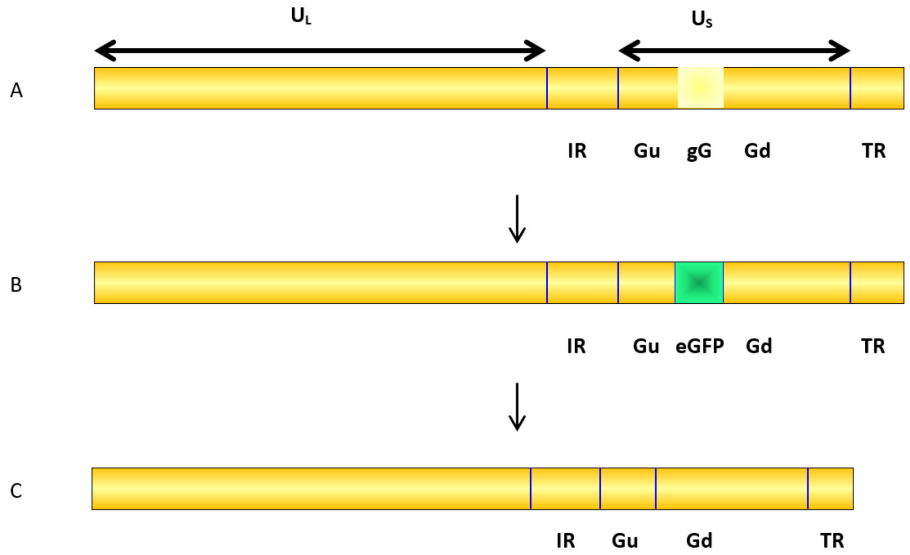

Figure 3: Construction of the GM virus. (A) Wild type ILTV genome with gG gene flanked by upstream and downstream sequences. (B) gG was replaced with eGFP resulting in ΔgG(eGFP) ILTV. (C) eGFP was removed from ΔgG(eGFP) ILTV genome resulting in ΔgG ILTV (the GMO). IR: internal inverted repeat. TR: terminal inverted repeat. gG: coding region of gG gene. Gu: US2, PK and UL47 genes upstream of gG. Gd: gJ, gD, gI, gE, and US9 genes downstream of gG. eGFP: enhanced green fluorescent protein. Adapted from Devlin et al. (2006).

68. The region of the GMO genome flanking the deletion was sequenced. The sequence data indicates that the gG transcription start and termination sequences are intact, and theoretically, transcription of an approximately 150 nucleotide mRNA could occur. As the translation initiation (ATG) codon remains in the transcript, this mRNA could theoretically result in the translation of a 27 amino acid non-functional protein corresponding to non-coding regions of the gG mRNA. The potential expression of the mRNA and protein has not been investigated.

69. Compared with the CSW-1 strain, the GMO also has a two base-pair deletion in the non-coding sequence four base pairs 5' to the initiation codon, and a single A to G transition in the non-coding sequence approximately 700 base pairs 5' to the initiation codon.

4.2 Glycoprotein G

70. Glycoprotein G (gG) is conserved in most members of the *Alphaherpesvirinae* subfamily. It is described as a viral chemokine binding protein (vCKBP) (Bendezu et al., 2019), which is secreted or anchored on the plasma membrane of the infected cell (Bryant et al., 2003). Studies of ILTV gG showed that it could be responsible for modulating the host inflammatory response and influencing the recruitment of immune cells to the site of infection (Coppo et al., 2018).

71. Various other *in vitro* studies have been carried out to determine the function of gG in alphaviruses (*Equine herpesvirus*, *Bovine herpesvirus*, *Herpes simplex virus* and *Feline herpesvirus*) (Tran et al., 2000; Nakamichi et al., 2001; Nakamichi et al., 2002; Bryant et al., 2003; Costes et al., 2005; Huang et al., 2005; Bendezu et al., 2019). These studies demonstrated various functions of gG (plaque formation; cell attachment; modulation of host immune response; replication and infectivity of the virus; and production profile).

4.3 Characterisation of the GMO

4.3.1 Growth kinetics and virulence

72. No significant difference was observed in the growth kinetics between the GMO and the CSW-1 parent strain *in vitro*. The removal of gG did not affect transcription of the upstream and downstream

sequences immediately adjacent to gG. The ability of the virus to spread cell-to-cell as measured in plaque assays was similar to CSW-1 (Devlin et al., 2006).

73. Chickens inoculated with the GMO also had greater tracheal mucosal thickness than those inoculated with CSW-1 ILTV or ΔgG(R) ILTV. The increase in mucosal thickness is consistent with increased inflammatory cell infiltrate in the mucosa. This suggests that gG may play a role in influencing the inflammatory response at the site of ILTV infection (Devlin et al., 2006).

74. The GMO was shown to be attenuated as chickens inoculated with the GMO showed milder ILT disease symptoms and had greater weight gain at 4 days post-infection compared to those inoculated with WT CSW-1 ILTV or with ILTV where the gG gene was reinserted (ΔgG(R) ILTV) (Devlin et al., 2006). In the same study, chickens inoculated with the GMO had similar titres of virus in the trachea as those inoculated with CSW-1 ILTV or ΔgG(R) ILTV, suggesting that the capacity for *in vivo* replication and shedding of the virus from the trachea was not affected by the loss of gG (Devlin et al., 2006).

75. The replication of ILTV DNA after eye drop administration of the GMO was carried out in two peer-reviewed studies (Coppo et al., 2011; Thilakarathne et al., 2019), one small-scale – Study 1 (5 chickens; 4 time points) and four large-scale field trial studies – Study 2 (3 sheds; 20 chickens (tracheal swabs) or 2 shed; 20 chickens or 3 sheds; 1400 chickens (palatine cleft swabs i.e. roof of the mouth) as summarised below. Note that for the large-scale studies a sample size of 10 (trachea/palatine cleft) and 15 (palatine cleft) were used for ILTV PCR detection and not every chicken was tested. A summary of the studies using eye drop administration are described below (Table 2):

Table 2 Summary of studies with the GMO.

Days post vaccination	Findings	Reference
4	<u>Detection in trachea</u> • Low level of detection (10%; 20 chickens) • No detection (0%, 5 chickens) • High level of detection (average of 70%; 3 groups of 10 samples/group) <u>Detection in palatine cleft (more sensitive detection)</u> • High level of detection (90%; 10 samples and 78%; 3 groups of 15 samples/group)	• Thilakarathne et al. (2019) • Study 1 • Study 2 • Study 2
7	<u>Detection in trachea</u> • No detection (0%; 5 chickens) • Low level of detection (average of 20%; 3 groups of 10 samples/group) <u>Detection in palatine cleft (more sensitive detection)</u> • Low level of detection (35%; 2 groups of 10 samples/group and 25%; 2 groups of 15 samples/group)	• Study 1 • Study 2 • Study 2
14	<u>Detection in trachea</u> • No detection (0%; 20 chickens)	• Thilakarathne et al. (2019)

	• No detection (0%; 5 chickens) • 1 positive detection from 3 groups of 10 samples	• Study 1 • Study 2
20 or 21	• No detection in trachea, conjunctiva, palatine cleft and infraorbital sinus • Detection of GMO DNA in trigeminal ganglia but no replicating ILTV detected likely due to low rate of infection and reactivation of ILTV in the neurons, suggesting infection could be latent. • Low level of detection in the trachea (9.5%; 21 chickens)	• Thilakarathne et al. (2019) • Coppo et al. (2011)

76. In summary, the data suggests that the peak of detection of GMO DNA is at 4 days post vaccination (dpv) in the trachea and palatine cleft, is minimal from 7 dpv but can be detected in the trigeminal ganglia at 21 dpv, suggesting potential latency of the virus. In addition, when compared to other vaccine strains (Serva, A20 and SA2), the presence of detectable ILTV DNA in GMO vaccinated chickens was much lower than the other vaccine strains at time points described above (Coppo et al., 2011; Thilakarathne et al., 2019).

77. The presence of vaccine DNA was also studied in chickens vaccinated by drinking water (average of 40,000 chickens in 8 different sheds). The study showed a low level of ILTV DNA in the vaccinated chickens at 4, 7 and 21 dpv. Note that the sample size collected for the PCR testing was small (10-15 samples). Overall, Study 2 determined that administration using eye drops is the more effective method of administering the vaccine.

78. Initial published data demonstrated that mortality rates in chickens inoculated via eye drop with the GMO were lower (12.5%) than for CSW-1 ILTV (31.25%) or for ΔgG(R) ILTV (37.5%) (16 chickens / group) (Devlin et al., 2006). The mortality rates of eye drop administration of the GMO (12.5%) were also compared to the A20 vaccine strain (6.25%) and SA2 strain (43.75%) (16 chickens/group) (Devlin et al., 2007). Subsequently, larger scale field trials with the GMO vaccine approved under DIR-154, showed an average of 4% mortality rate in GMO vaccinated chicken (via drinking water) and normal weight gain. Based on the data, the applicant has suggested that the lower mortality rate could also be attributed to the low GMO uptake from the administration via drinking water.

79. To meet the *European Pharmacopoeia monograph 04/2013:50206 Evaluation of safety of veterinary vaccines and immunosera 5.2.6* and the *European Pharmacopoeia monograph 04/2013:1068 Avian Infectious Laryngotracheitis Vaccine (Live) for the demonstration of safety of a live vaccine in the target species,* additional studies were carried out with the GMO (master seed stock - MSV). The studies assessed the safety of the *Good Manufacturing Practice* (GMP) vaccine product under the Good Laboratory Practice (GLP) and the Asia Pacific Centre for Animal Health (APCAH) Quality policy (MM/QP/23). The unpublished studies showed that chickens receiving 10 times the dose or receiving the normal dose 3 times (14 days apart) via eye drop administration, did not show any clinical signs, mortality or tracheal pathology. In this study, the vaccine was not detected (by PCR) in any samples collected beyond 7 dpv (tracheal mucus, conjunctival swab, palatine cleft, feather pulp and both trigeminal ganglia).

80. The initial safety studies by Devlin et al in 2006 were carried using an experimental grade of the GM vaccine (earlier passage level than the MSV), which could explain the variation in mortality observed compared to later studies. The MSV stocks were subsequently used to manufacture the final GM product.

4.3.2 Bio-distribution, shedding and transmission

81. As mentioned above, the GMO DNA is mainly detected in the trachea of chickens, which is the main site of ILTV replication. To study transmission of the GMO, chickens that had been inoculated with either the GMO or CSW-1 ILTV (non-GM parent strain) 4 days earlier were introduced into cages with naïve chickens for 6 days. Both the GMO and CSW-1 were able to be transmitted to naïve chickens 6 days following exposure (26.7% GMO and 13.3% CSW-1) (Devlin et al., 2011).

82. No detectable ILTV DNA was observed in dust samples collected from GMO vaccinated and unvaccinated sheds taken at 14, 21, 28 and 33 dpv. Dust from farms following ILTV outbreaks (positive control), showed positive PCR readings, indicating the presence of ILTV.

83. Transmission of the GMO to naïve chickens was studied by the introduction of unvaccinated chickens into sheds with vaccinated chicken the morning after vaccination as part of Study 2 described above (20 naïve chickens to around 40, 000 vaccinated chickens / shed: 5 different sheds, chickens vaccinated via drinking water). Samples from naïve chickens were collected at day 7 and 14 after their introduction to allow time for transmission to occur. There was only one instance of transmission that was observed in the study (in one shed). These data show a very low transmission rate to in-contact birds. The applicant has suggested that the positive sample could also be attributed to potential exposure of the naïve chicken to residual vaccines in the drinking water line. In the same study it was shown using PCR that the vaccination rate was low (4 dpv – 27.5% tested chickens were infected with the vaccine strain. By 7 dpv – 12% tested chickens were infected; and this dropped by 14 dpv – 4% tested chickens were infected). Note that a sample size of 10 chickens were tested from each shed. The low detection of ILTV in the naïve introduced chickens may be attributed to the low numbers of chickens who were initially vaccinated via the drinking water in the shed. If high transmission of the GMO were to occur, the detection of ILTV would increase on Days 7 and 14 post vaccination as the GMO was transmitted between birds. Therefore, these data suggest that transmission of the GMO in sheds is possible but unlikely. Transmission of the GMO to susceptible bird species other than chickens has not been studied.

4.3.3 Phenotypic and genomic stability

84. The stability of the GMO's attenuation following transmission from ILTV infected chickens was determined using loss of weight as a proxy of ILTV infection. Naïve chickens were weighed before they were housed in the same cages as the GMO-inoculated chickens and at the end of the transmission study (Devlin et al., 2011). Weight gain of naïve, in-contact chickens that became infected with the GMO was not significantly different to naïve, in-contact chickens that did not become infected ($p = 0.281$ Student's t-test). This suggests that the GMO remained attenuated.

85. European Pharmacopoeia 04/2013:1068 (2.4.3) requires a test for increased virulence following sequential passage in birds. As mentioned in Section 4.3.2, transmission of the GMO to naïve chickens was rarely observed. Therefore, an unpublished study (done in accordance with GLP and APCAH) using manual transmission was done to assess whether sequential passaging could contribute to increased virulence. Ten chickens were first inoculated (eye drop) then euthanised at 4 dpv, where the tracheal mucosa was collected and tested for the GMO. If any virus was recovered, samples were used to inoculate (eye drop) the next 10 chickens and the process repeated five times. The study showed that the GMO was detected in the first group that were vaccinated. GMO samples were then pooled and used to vaccinate the second group of chickens. No GMO was detected in the subsequent groups.

86. Another unpublished study was carried out to determine the potential for the GMO to recombine with another field strain (V1-99) or a vaccine strain (Serva). Chickens were inoculated via eye drop with V1-99 alone, V1-99 + Serva and V1-99 + GMO. Samples were then collected at 2 dpv (tracheal and conjunctival) and 4 dpv (tracheal scrapings). DNA from the samples were then sequenced and recombination detected using the Recombinant Detection Program 4 (RDP4) as described in Martin et al. (2015). No recombination was detected using RDP4 in all the samples sequenced. However, these results

contrast with results described in Section 3.4, by Loncoman *et al* (V1-99 and CSW-1) and Fakhri *et al* (Serva and A20). In these studies, a different method to detect recombination events was used (single nucleotide polymorphism analysis) to detect recombination events after cells (Loncoman et al., 2017; Fakhri et al., 2020); chickens (Loncoman et al., 2017) or eggs (Fakhri et al., 2020) were co-infected with 2 different ILTV strains.

4.3.4 Efficacy

87. The efficacy of the GMO in protecting chickens from ILT disease was compared with other live attenuated vaccine strains, A20, SA2 and Serva (Coppo et al., 2011). Each treatment group, comprising 20 or 21 chickens, was inoculated with the vaccines 21 days prior to challenge with CSW-1 ILTV. Five days after challenge, chickens were clinically scored by determining their demeanour, breathing and incidence of conjunctivitis. On average, chickens inoculated with the GMO showed normal demeanour, no laboured breathing and no signs of conjunctivitis. No significant differences in the median clinical scores were observed between chickens inoculated with the GMO and the A20, SA2 and Serva group ($p > 0.05$, Student's t-test).

88. At 6 days after challenge, the chickens were sacrificed. Each chicken was weighed to calculate the weight gain, and tracheal histopathology was examined. The weight gain of chickens inoculated with A20 vaccine was the highest of all the groups (27.5% in males and 22.5% In females), but was similar between the SA2, Serva and the GMO groups (around 10% for both males and females). Chickens challenged after vaccination showed a low tracheal histopathology score, which is generally consistent with an immune response rather than acute viral replication and this observation was similar in the different vaccine groups (Coppo et al., 2011).

89. Subsequent studies with the GMO demonstrated that birds vaccinated via drinking water (Korsa et al., 2015) and eye drops (Korsa et al., 2018) were protected from subsequent challenge with Class 9 ILTV (last known common circulating strain in Australia) compared to unvaccinated birds. The administration via eye drop was shown to be more effective in protecting chickens that were subsequently challenged with Class 9 ILTV than chickens vaccinated via drinking water (Korsa et al., 2015; Korsa et al., 2018).

4.3.5 Decontamination of the GMO

90. Methods to decontaminate ILTV, which has been described in Section 3.5.6, would also be effective against the GMO.

Section 5 The receiving environment

91. The receiving environment forms part of the context for assessing risks associated with dealings with GM vaccine (OGTR, 2013). It informs the consideration of potential exposure pathways, including the likelihood of the GMO spreading or persisting outside the site of release. Relevant information about the receiving environment includes state and local council requirements relevant to poultry farming; commercial farming and processing practices; biosecurity standards for poultry farms; waste management practices; related viral species in the environment; and potential hosts in the environment.

5.1 Site of vaccination

92. The intended primary receiving environment would be the chicken, either via the conjunctiva (the eyes) as the GM vaccine will be delivered as eye drops, in drinking water, in eggs or by coarse spraying. Administration of the vaccine by drinking water and eye drop is currently authorised under an APVMA permit and DIR-154. To use the other methods of administration (i.e. in eggs and coarse spraying), the applicant would also need to seek a permit from the APVMA to authorise these modes of administration.

93. The secondary receiving environment would be the poultry farms where the chickens would be vaccinated.

94. The principal route by which the GMO may enter the wider environment following vaccination is via shedding. Further, GMO may also enter the environment via accidental spills of the vaccine, residual GMO on the surface of feathers (coarse spraying) or in eggs (*in ovo* inoculation).

5.2 Corporate structures

95. The chicken meat industry is predominantly vertically integrated, where generally, individual companies own almost all aspects of production (breeding farms, multiplication farms, hatcheries, feed mills, some broiler farms, and processing plants). Two large integrated national companies supply more than 70% of Australia's broiler (meat) chickens - Baiada and Inghams Enterprises. Inghams and Baiada are privately owned, with farming and processing operations in most states. The rest are medium-sized, privately owned companies, and a number of smaller processors.

96. Growing broiler chickens, from one day old chicks to the day of processing, is generally contracted out by processing companies to contract growers. Approximately 800 growers produce about 80% of Australia's broiler chickens under these contracts. Other broiler chickens are produced on large company farms, or on farms owned and managed by 'intermediary' companies which own a number of farms, each managed by a farm manager, and who enter into contracts with processing companies to grow out chickens on a larger scale.

97. Contract growers own the farm and provide the management, infrastructure, equipment, labour, bedding and other inputs to rear chickens. The processing company provides (and owns) the chickens and provides feed, medication and technical advice (Australian Chicken Meat Federation, 2020).

98. Limited publicly available information is available on the corporate structure of the egg production industry, but the information available suggests a similar structure to the chicken meat industry (Australian Eggs, 2022a).

5.3 Poultry farm management

99. Poultry farms need to comply with a range of state and territory requirements designed to protect people and the environment (see Table 3). Farms should also adhere to quality management systems incorporating standards such as GMP and the Hazard Analysis of Critical Control Points (HACCP), and manage the farms in accordance with strict state environmental codes.

Table 3 Links to state and territory requirements in Australia.

State	Website
ACT	https://www.accesscanberra.act.gov.au/s/article/environment-protection-guidelines-tab-overview
NSW	https://www.dpi.nsw.gov.au/animals-and-livestock/poultry-and-birds
NT	https://nt.gov.au/industry/agriculture/livestock/keeping-poultry-and-pigeons*
QLD	https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/livestock/poultry/poultry-farming-queensland/starting-poultry-farm/legal-requirements
SA	https://www.pir.sa.gov.au/biosecurity/animal_health/poultry
TAS	https://nre.tas.gov.au/biosecurity-tasmania/product-integrity/food-safety/meat-and-poultry

VIC	https://agriculture.vic.gov.au/livestock-and-animals/poultry-and-eggs/compliance/laws-regulations-and-standards-for-poultry-owners
WA	https://www.agric.wa.gov.au/livestock-biosecurity/regulation-poultry-production
*No large commercial poultry farms are currently present in NT	

100. Local councils and/or state government agencies such as Environment Protection Authorities (EPA) are responsible for the approval of intensive agriculture developments including free range poultry farms. Local councils are generally the responsible authority for the administration or enforcement of planning schemes. This means that councils would assess and determine farm planning permit applications. Councils are also responsible for monitoring and enforcing the compliance of poultry farm operators with their planning permit conditions. In addition, the poultry company may require minimum distances between the poultry farm and other poultry farms, or livestock farms owned or managed by them or by others.

101. Boundary setbacks may be required by councils and are defined as the distance between the nearest external edge of any new chicken shed, litter stockpile or compost pile and the farm boundary. Boundary setbacks mitigate visual amenity issues and the immediate impact of odours, dust, aerosols and noise emissions from sheds, litter, or compost piles on the amenity of adjacent land and the surrounding area.

102. Separation distances are used to reduce the effects of odour, dust, aerosols and noise of a chicken farm. The separation distance is the distance from the nearest external edge of a broiler shed to the nearest external edge of a sensitive use (e.g. house or public building) on land beyond the broiler farm property. It excludes sensitive uses directly associated with the broiler farm operations – e.g. residential dwellings on the broiler farm property. Separation distances usually extend across adjoining properties that are not owned by the farm owner. Where separation distances are not specified by state and local government departments and agencies, the following separation distance are suggested: 500 m separation between farm and land zone that is not compatible with development (e.g. residential/rural residential areas) and 250 m separation between farms and any sensitive land use (e.g. neighbouring houses) located on land that is compatible with development (e.g. on land designated rural, farming or similar) (McGahan, 2021). A guideline published by AgriFutures also included a separation distance formula, which considers number of birds, location, terrain and climate (McGahan, 2021). The greater the separation distance and the boundary setback, the lower the probability of offensive odour and dust adversely impacting the surrounding community.

103. Buffer zones would be used to separate the poultry sheds from adjoining developments. The farm owner has legal control of the buffer zone. A buffer may be open farmland, or a landscape area that hides views of the sheds or helps to disperse odours.

5.3.1 Broiler chicken farms

104. When commercial chickens are grown for meat, they are commonly referred to as broilers and can be grown in conventional, free-range and organic production systems. Australian broilers are not kept in cages, regardless of the production system used (Poultry Hub Australia, 2022d).

105. Commercial chickens used as broilers are transported from hatcheries to broiler farms in ventilated chick boxes in air-conditioned trucks prior to going through the following phases:

- brooding phase, where chicks are placed on the floor of the sheds and given supplementary heating;
- growing phase (around 42-56 days of age); and
- harvesting phase (when chickens are transported to a factory for processing).

106. During transport for processing, chickens are placed in crates in an open truck and transported in accordance with the relevant state legislation. Crates, trucks, equipment and other materials used to transport the chickens from the shed to the processing plants are decontaminated with disinfectant after delivery of chickens. The Standard for Poultry Meat (Standard 4.2.2) requires that transportation vehicles and equipment be effectively cleaned, sanitised and in good working order to ensure poultry is not made unsafe or unsuitable for human consumption (Food Standards Australia New Zealand, 2012).

107. The sheds are cleaned out once all the birds are harvested (approximately 60 days) and prepared for a new batch of chicks. This involves removing bedding, brushing floors, scrubbing feed pans, cleaning out water lines, scrubbing fan blades and other equipment, and checking rodent bait stations. High-pressure hoses are used to thoroughly clean the whole shed. The floor is usually rammed earth and because low water volumes are used, there is little water runoff. Once cleaned, the sheds are sanitised with disinfectant or insecticides that have been approved by the APVMA. After a full clean-out, company veterinarians or servicemen will test the shed to confirm that it has been adequately cleaned and that any potential disease agents, including ILTV are removed (Poultry Hub Australia, 2022e).

108. Free range broiler chickens are produced using similar management, housing, rearing and feeding practices as conventional broiler chickens. Free range broiler chickens are harvested within the same timeframe as shed-housed chickens. The major differences are that free range broiler chickens are allowed access to an outside run for part of each day (at least after the brooding period) and often have lower target stocking densities.

109. Only free-range chickens can be used to produce certified organic meat and must be grown without the use of artificial colours and synthetic chemicals; be fed predominantly certified organic ingredients; and cannot be treated with routine vaccination unless it is required by law or if the disease cannot be controlled with organic management practices (Poultry Hub Australia, 2022a). ILTV vaccination is currently not a routine vaccination for chickens.

5.3.2 Layer chicken farms

110. When commercial chickens are grown to produce eggs, they are commonly referred to as layers and can be grown in caged, barn laid, free range and organic production systems. Production can range from extensive (small scale), semi-intensive (few hundred to thousands of hens) and intensive (100, 000 – 500, 000 hens) (Poultry Hub Australia, 2022b).

111. Commercial chickens used as layers typically go through the following phases (Poultry Hub Australia, 2022c):

- The brooding phase (day old-6 weeks), where they need additional heat to control their body temperature.
- The growing phase (6-20 weeks), where they can regulate their body temperatures but still need to be protected from climate extremes.
- The moving phase (16-18 weeks), when the hens are moved to their laying quarters.
- The adult layer phase (20 up to 78 weeks), where they are producing eggs. They would need to be fed carefully and housed at 21-28°C.

112. Caged farming relies on the layers being kept in cages within sheds that include automated feeding, watering and climate control, ventilation and lighting. The Model Code of Practice for the Welfare of Animals stipulates the minimum space between hens is 550 cm^2 (Australian Eggs, 2022c).

113. Layers that produce barn laid eggs are allowed to roam the sheds without being in kept in cages (Australian Eggs, 2022b).

114. Free range layers are allowed meaningful and regular access to an outdoor range during daylight hours (Australian Eggs, 2022d).

115. Similar to broilers, organic eggs can only be produced by free range layers that must be raised without the use of artificial colours and synthetic chemicals; be fed predominantly certified organic ingredients; and cannot be treated with routine vaccination unless it is required by law or if the disease cannot be controlled with organic management practices (Poultry Hub Australia, 2022a).

5.4 Biosecurity

116. Each state and territory have their own biosecurity regulations and legislation. The following state departments are responsible for the biosecurity for each state and territory:

- Environment, Planning and Sustainable Development Directorate – Environment (ACT);
- Department of Primary Industries (NSW);
- Northern Territory government (NT);
- Department of Agriculture and Fisheries (QLD);
- Department of Primary Industries and Regions (SA);
- Department of Natural Resources and Environment Tasmania (TAS);
- Agriculture Victoria (VIC); and
- Department of Primary Industries and Regional Development (WA).

117. Biosecurity considerations includes the following: the identification of livestock and commercial farms; management of animal diseases (including vaccination and risk of transmission); animal welfare; controls on feeding livestock and transport of livestock. Australia also has a national *Biosecurity Act 2015* and a website for managing and reporting national pest and disease outbreaks, that is managed by the Department of Agriculture, Fisheries and Forestry (DAFF).

5.5 Poultry farm biosecurity standards

118. There are also various guidelines published by Animal Health Australia (AHA) and DAFF in conjunction with various poultry industry groups and state and territory departments. This includes the *National Farm Biosecurity Manual Poultry Production*, *National Water Biosecurity Manual Poultry Production* and *National Farm Biosecurity Technical Manual for Egg Production* (Department of Agriculture Fisheries and Forestry, 2009a, b; Animal Health Australia, 2020). These guidelines include documentation and training; facility standards; personal protection equipment and procedures; operational standards and high-risk biosecurity procedures. These standards are applicable to all poultry producers including free range farms. There are 2 levels of biosecurity, Level 1 (routine biosecurity that should be implemented and followed daily) and Level 2 (high risk biosecurity that should be implemented in the event of an outbreak of an emergency or serious endemic disease) (Department of Agriculture Fisheries and Forestry, 2009a).

5.5.1 Documentation and training

119. Under these guidelines, each production facility must keep a copy of the *National Farm Biosecurity Manual Poultry Production* and/or the *National Farm Biosecurity Technical Manual for Egg Production* depending on the type of facility or a more detailed document encompassing either manual, that is easily accessible to staff. The manual also states that staff need to be trained in relevant parts of the manual and evidence of this training kept.

5.5.2 Facility standards – conventional and free range

120. The guidelines also states that the production area (sheds or free-range area, feed storage and handling area, and area immediately surrounding the sheds including pick-up areas) must have a perimeter fence or otherwise well-defined boundary (e.g. creek, vegetation) establishing a clearly defined biosecurity zone. Trees and shrubs should be selected to minimise wild bird attraction. The area

around sheds must be kept free from debris and vegetation should be mown regularly. Vegetation buffers for environmental compliance should not be compromised.

121. The production area must have a stock proof fence If livestock graze on the property. Grazing near sheds (i.e. on part of the production area) is only permitted where the grazing area is separated by a stock proof barrier from the area used by poultry. Drainage from livestock pastures must not enter poultry enclosures or areas that can be accessed by poultry. These standards are in place to prevent transmission of contaminants from livestock to poultry.

122. The main entrance to the production area must be capable of being closed to vehicle traffic (e.g. lockable gate which should be kept locked at all times) and must display appropriate signage including 'Biosecure Area No Entry Unless Authorised' or similar wording. In addition, signage including contact numbers must direct visitors to contact the producer before proceeding. Facilities should be available for the cleaning and disinfection of equipment before entry.

123. There must be a change area away from sheds with clean protective clothing and boots provided. Entry to sheds must only be made through entrances with a footbath containing a suitable disinfectant. There must be provision for scraping the soles of boots before dipping to ensure the disinfectant contacts the soles of the boots. An alternative system using separate production area- and shed-footwear may be used. Facilities for hand sanitation must also be placed at the entry to each shed.

124. Feeding systems must, wherever possible, be closed to ensure that feed in silos and feed delivery systems are protected from access and contamination by wild birds and rodents. Feed spills should be cleaned up without delay to prevent the congregation of wild birds.

125. Drinking water should be accessed inside the shed; or, if watering stations are required outside, they should be of a type that cannot be easily accessed by wild birds (e.g. a nipple system). The watering system should be maintained, to prevent leakage and the creation of wet patches within or outside the shed. Water tanks should be checked regularly to ensure that they remain bird-proof.

126. Drinking water for poultry, as well as cooling water used in poultry sheds, must meet appropriate water standards. Water that does not meet the standard must be treated (e.g. chlorination, ultraviolet, iodine) to ensure that the standard is met. All surface water (dam, river etc.) must be treated before being used as drinking water for poultry. Treated water supply must be kept in a closed system from the point of treatment to the drinker.

127. All poultry housing must be designed and maintained to prevent the entry of wild birds and limit the access of vermin as far as is practical.

128. The production area should be adequately drained to prevent accumulation and stagnation of water likely to attract waterfowl, especially in the areas around sheds.

129. An appropriate vermin control plan must be developed and implemented, including rodents, foxes, wild dogs and cats. A baiting program for rodents must be implemented where a risk assessment deems this necessary (e.g. live rodents, droppings, nests). Beetle populations within shed litter should be controlled via an integrated pest management approach by using pesticides, composting and total shed and litter clean-out.

130. Only commercially produced avian species are to be kept in the production area and no other avian species (including aviary birds and pet birds) or pigs are to be kept on the property.

131. If more than one commercially produced avian species is kept in the production area, the species should be housed and managed separately, with suitable biosecurity arrangements for each species. Shared equipment should be cleaned and disinfected between uses.

132. Used litter and manure must not be stockpiled in the production area. Used litter and manure must be stored in an appropriately designed storage area away from the production area.

133. Dead chickens must be collected regularly and stored in freezers if the number of dead birds is likely to cause environmental impacts or increased biosecurity risks. Containers and freezers used for collecting dead birds must be cleaned and sanitised between batches. Dead bird disposal methods must conform with applicable environmental compliance requirements.

5.5.3 Additional standards for free-range farms

134. The following biosecurity measures are specific for free-range farms in addition to the conventional farming described above.

135. Good fencing is required to prevent the entry of animals such as dogs, foxes and cats. In many situations, however, fencing alone is insufficient to stop such intrusions; therefore, some free-range enterprises keep specially trained dogs with the chickens, as protection against other animals and against unauthorised human entry. Dogs must not enter sheds unless as part of the flock security strategy. Guard dogs such as these are not regarded as a biosecurity risk but rather as a biosecurity tool.

136. Where footbaths are not appropriate for a free-range paddock, a system should be documented and implemented to monitor and prevent any potential hazardous organic material or litter entering free range paddocks.

137. In free range farms, chickens may have some exposure to wild birds. Therefore, documented measures must be taken to minimise the congregation of waterfowl and the impact of wild birds. The attraction of wild birds can be minimised by placing feeders and water inside the shed, rather than in the open range where wild birds would have easier access. Placement of bird netting in critical feeding areas may also reduce the risk.

138. In free-range farms with sheds or other housing, manure deposits outside the hatch openings must be removed after each batch, and ramps used by chickens must be scraped and cleaned after each batch.

139. Grass on and around the farm must be kept cut to reduce rodent attraction.

5.5.4 Farm personnel and visitor standards – conventional and free range

140. Production area personnel or any person residing on the property must not have contact with any other poultry, avian species or pigs unless they have a complete head-to-toe shower and change into new protective footwear and clothing prior to entering the production area.

141. Personnel must wear laundered clean clothes each day to work and ensure that they do not become contaminated by contact with avian species or pigs on their way to work. It is critical that boots worn in sheds are not worn or taken outside the production area.

142. Company service personnel visiting the production area must wear protective clothing and footwear, as approved by the production facility manager. Hands must be sanitised before entering sheds.

143. Contractors who have had contact with poultry or other birds that day or keep birds at their home must not enter sheds and/or ranges populated or ready to be populated with birds unless it is an emergency, and they have showered from head-to-toe, changed clothes and boots and wear hair covering. Tools taken into the production area must be cleaned before entry into sheds and must be free of dust and organic matter.

144. All persons must agree to comply with the entry conditions by signing the visitors' log and such visits must be approved by the manager before visitors may enter sheds and ranges. This requirement also applies to vaccination crews.

145. During processing of chickens, pick-up crews work from youngest to oldest or all young birds or all old birds on a shift basis in accordance with the processing company's pick-up biosecurity procedures. Pick-up crews must not keep birds at their homes. Drivers must sanitise their hands and boots before

and after each pick-up or delivery to a production area. Trucks carrying unused or used litter must be cleaned and disinfected between production areas.

146. A system for tracing movements of delivery personnel (e.g. through delivery dockets and feed company records) must be implemented.

5.5.5 High level biosecurity

147. In the event of an outbreak of disease, the *National Farm Biosecurity Manual for Poultry Production* recommends the following measures (Department of Agriculture Fisheries and Forestry, 2009a):

- limiting visitors from entering the production area unless essential;
- visitors must have a head-to-toe shower before and after visit;
- used clothing and personal protective equipment must remain on property;
- any vehicle entering the property must be washed and disinfected before and after going onto the property;
- poultry and litter must not be moved on or off property until disease status is clarified.

148. Farms require a contingency plan to cope with occurrences of high mortalities. An investigation must be conducted to ascertain the cause of death and the best option for the disposal of the dead birds. Where normal disposal methods are not feasible, the relevant regulatory authorities (e.g. the local council, the state EPA) may need to be contacted to help identify alternative options.

149. If the cause of the death is an Emergency Animal Disease, then the relevant Australian Veterinary Emergency Plan (Ausvetplan) would be activated, and the appropriate authorities would be notified. In this situation, the entire flock may be euthanised. The disposal of carcasses, used litter and feed, and decontamination of equipment, would be under the direct control of the state's Chief Veterinary Officer.

150. The Biosecurity Incident Management System provides guidance for the management of biosecurity incident response in Australia and can be applied to all biosecurity sectors. Typically, the states and territories have primary responsibility for preparing and responding to biosecurity incidents within their borders. DAFF has a role in providing national leadership and coordination in preparing for and responding to biosecurity incidents.

5.6 Transport of live chickens

151. In Australia, transport of animals including poultry is regulated by state and territories in accordance with the *Australian Animal Welfare Standards and Guidelines – Land Transport of Livestock* (Animal Health Australia, 2012). The standards cover responsibilities and competency of personnel; transport vehicles and facilities (e.g. temperature, ventilation and containers, including cleaning processes); loading, transporting, and unloading procedures; and the humane destruction of livestock.

152. The standards apply to all those responsible for the care and management of livestock that are transported, including drivers, transport companies, owners, agents and livestock handlers at farming enterprises, depots, saleyards, feedlots and livestock-processing plants. The chain of responsibility begins with the owner or their agent and extends to the final receiver of the livestock (Animal Health Australia, 2012).

153. Prior to transport, poultry stocks are assessed to determine if they are suitable for transport by the grower. Any birds that are found to be unsuitable for transport are managed on farm or humanely destroyed before the day of pick up (Animal Health Australia, 2012).

154. The implementation dates for the standards by the states and territories can be found here. In addition, each state and territory are also responsible for the regulation of animal welfare. A list of the state and territory animal welfare legislation can be found here.

5.7 Waste management

155. The management of waste (litter and carcasses) from poultry farms must meet the requirements of the Environment Protection Authority (EPA) from the different states and territories in addition to local councils. This management strategies include composting, burial on the farm/landfill and transport to rendering farms, which have been described in detail in the RARMP for DIR-154.

156. In brief, litter/waste must:

- be removed immediately and transported in covered vehicles;
- be managed to avoid contamination of surface waters, stormwater drains, waterways, catchment and ground waters, and avoid excessive fly breeding;
- not be buried near houses and water sources; and
- be kept free of rodents, cats, dogs, feral animals, scavenging birds and flies.

157. Chicken carcasses could be sent to rendering farms where they are processed with high heat and pressure. The rendering facilities are regulated by state/territory or local council requirements. They are highly automated, minimising any direct contact of workers or the external environment with microbiological contaminants. The high heat and pressure used in the rendering process would destroy any GM vaccine that are potentially still present in the carcasses.

5.8 Presence of related viral species in the receiving environment

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

159. Three live attenuated ILTV vaccines are registered for use in chicken farms in Australia. From 2007-2015, ILTV outbreaks in NSW and Victoria were caused by different classes of ILTV including those originally derived from the vaccine strains (see Section 3.5.4). ILT disease continues to be a problem in Australia with reports of ILT disease in VIC in 2016 and NSW and SA in 2019. The National pest and disease outbreaks website, has no current listed outbreaks of ILTV (as of Oct 2022)

160. Another virus belonging to the subfamily *Alphaherpesvirinae* that commonly infects poultry, including chickens is *Gallid herpesvirus* type 2 (Marek's disease virus). Marek's disease affects both commercial and backyard poultry and is endemic in Australia.

161. *Psittacid herpesvirus 1* (PsHV-1) also belongs to the *Iltovirus* genus in the subfamily *Alphaherpesvirinae*. PsHV-1 causes Pacheco's disease, an acute and potentially lethal respiratory infection in psittacine birds including macaws, parrots and cockatoos. Based on sequence analysis of PsHV-1 and ILTV, these viruses are phylogenetically closely related. The similarity of their genomes suggests that they represent a class of avian alphaherpesviruses that diverged early from a common ancestor and are distinct from the Marek's disease virus (Thureen and Keeler, 2006). PsHV-1 and ILTV do not share the same host species. PsHV-1 has not been reported in wild bird populations in Australia and has only been reported in very rare cases from birds imported from overseas (Wildlife Health Australia, 2017; Department of Agriculture Fisheries and Forestry, 2020).

162. There are also several other avian herpesviruses known but these are not known to infect chickens as herpesviruses tend to be host specific.

5.9 Presence of similar genetic material in the environment

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

164. The GMO was derived from naturally occurring ILTV isolated in Australia, hence similar genetic material would already be present in the environment.

5.10 Potential hosts in the environment

165. The potential for ILTV to infect other susceptible hosts that may be present at or near the proposed trial sites is considered in the risk assessment (Chapter 2). The primary host for ILTV is the chicken. ILT disease in turkeys, pheasants and peafowl is rarely reported (see Chapter 1, Section 3.5.1). Throughout its long history since its initial reports in various parts of the world, ILTV outbreaks have occurred mostly in chicken farms.

166. Other birds such as ducks may act as carriers of ILTV, but there is limited evidence of their role in spreading the virus and attempts to infect ducks were unsuccessful (see Section 3.5.1).

167. Australia has feral chickens, turkeys, pheasants and peafowls, from the family *Phasianidae*. A search in the Australian Bird & Bat Banding Scheme (ABBBS) database showed that Australia has 3 native species belonging to *Phasianidae* family *(Coturnix chinensis*- King Quail*, Coturnix pectoralis* – stubble quail*, and Coturnix ypsilohora* – brown quail) and 2 introduced species (*Pavo cristatus* – Indian peafowl and *Phasianus Colchicus* - common pheasant). As described in Chapter 1, Section 3.5.1, quail species are found to be resistant to ILTV infection. However, peafowls and pheasants can be susceptible to ILTV infection. Indian peafowl and common pheasant are introduced species, not commonly found, and are listed as feral pests in Australia (West, 2011).

Section 6 Previous authorisations

168. This GM vaccine has not been previously authorised for commercial supply in any region or country. The APVMA has issued a permit for the use of the GM vaccine for research only.

169. Work to develop the GMO in the laboratory including testing and preliminary experiments was conducted by the University of Melbourne and Royal Melbourne Institute of Technology (RMIT). The vaccine is manufactured under NLRD-10741.

170. The Regulator has issued one DIR licence (DIR-154) for field trial experiments in relation to this GM vaccine.

Chapter 2 Risk assessment

Section 1 Introduction

171. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

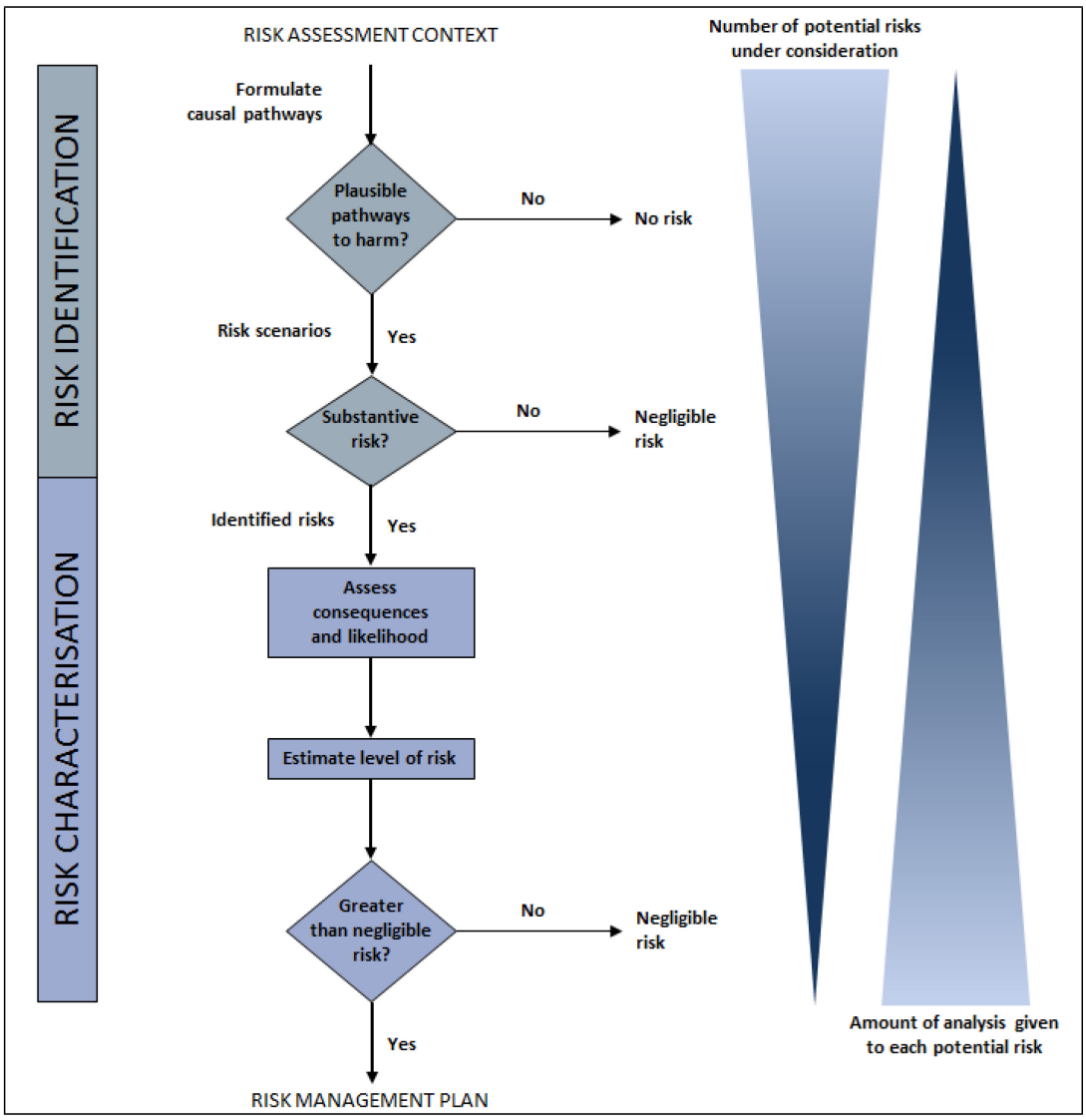

Figure 4: The risk assessment process

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

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

174. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 4), i.e. the risk is considered no greater than negligible.

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

Section 2 Risk identification

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

i. The source of potential harm (risk source)

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

iii. Potential harm to people or the environment.

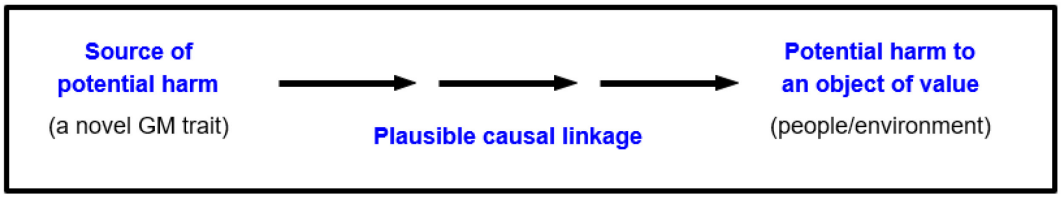

Figure 5: Components of a risk scenario

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

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

2.1 Risk source

178. The parent organism is an infectious laryngotracheitis virus (ILTV) CSW-1 strain. Details of the pathogenicity and transmissibility of ILTV is discussed in Chapter 1. Infection is generally the result of direct contact of mucosal secretions containing the virus. Chickens vaccinated with the GMO could transmit the GMO to uninfected chickens or other susceptible avian species (e.g. turkey, pheasants and peafowl).

179. The sources of potential harms can be the intended novel GM traits associated with the deletion, or unintended effects arising from the use of gene technology.

180. As discussed in Chapter 1, Section 4.1, the GMO has been modified by deleting the *gG* gene resulting in an attenuated virus. This modification is considered further as a potential source of risk.

181. Unintended effects can arise through horizontal gene transfer (HGT) which is the stable transfer of genetic material from one organism to another without sexual reproduction. As discussed in Chapter 1, Section 4.3, there is a possibility that the GMO can revert to the WT pathogenic strain or could recombine with other field or vaccine strains resulting in a novel trait. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism. This pathway is further considered as a potential source of risk.

182. Infection with ILTV could result in latent infection in the birds' trigeminal ganglia and increase the period of viral persistence and transmission. Therefore, this pathway is further considered as a potential source of risk.

183. ILTV is not known to integrate into the host DNA as discussed in Chapter 1, Section 3.3. Thus, the consequences of integration of viral DNA into a host cell genome will not be further discussed.

184. As discussed in Chapter 1, Section 4.1, sequencing of the region of the genome from which the gG gene was deleted indicates that there is a theoretical potential for a novel 150 nucleotide mRNA transcript from across the deletion site, encoding a 27 amino acid protein. The potential expression of the mRNA and protein and whether it could be toxic or allergenic has not been investigated. The genetic sequence is not unique and is part of the parent ILTV strain. It is very unlikely that the protein that could potentially be expressed could lead to any toxic or allergenic reactions. Therefore, the potential risks of the expression of 27 amino acids causing toxicity or allergenic reactions will not be further discussed.

185. ILTV is known to have a very limited host range and not shown to be able to infect and cause disease in non-avian species. In the event of exposure of other animals to the GMO, it is highly unlikely that it would lead to any harm to those animals. Therefore, this pathway will not be further discussed.

186. The current assessment focuses on risks posed to people and to the environment, including long term persistence of the GMO, which may arise from the transport, storage or disposal of the GMO.

2.2 Causal pathway

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

- the proposed dealings, which are transport or disposal of the GMO and possession (including storage) in the course of any of these dealings,
- regulations in placed for the transport or disposal of the GMO by other regulatory agencies, the States and Territories,
- characteristics of the parent organism,
- routes of exposure to the GMOs,
- potential for transmission,
- potential effects of the deleted gene on the properties of the organism,
- potential exposure of other organisms to the GMOs in the environment,
- the release environment,
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential),
- environmental stability of the organism (e.g. tolerance to temperature, UV irradiation and humidity),
- potential risk of revertant/novel strains due to HGT,
- practices before and after administration of the GMO including commercial poultry farming practices.

188. Although these factors are taken into account, some are not included in the risk scenarios because they are regulated by other agencies, have been considered in previous RARMPs or are not expected to give rise to substantive risks (see Sections 2.4.1 to 2.4.3 below).

189. The APVMA regulates the quality, safety and efficacy, and trade risks associated with the GM vaccine under the AgVet Code, as mentioned in Chapter 1, Section 1.1. This includes safety and efficacy of the vaccine; environmental risks; and recommended practices for the use, transport,

storage and disposal of the GM vaccine. Therefore, risk scenarios in the current assessment focus primarily on risks posed to people and to the environment from the GMO, and not the intended vaccine recipients (chickens).

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

2.3 Potential harms

191. Potential harms from the GM vaccine include:

- harm to the health of people or desirable organisms, including disease in humans or birds or adverse immune response to the GMO
- the potential for establishment of a novel virus that could cause harm to people or the environment.

2.4 Postulated risk scenarios

192. Four risk scenarios were postulated and screened to identify substantive risk. These hypothetical scenarios are summarised in Table 4.

193. In the context of the activities proposed by the applicant and considering both the short and long term, three of the four risk scenarios did not give rise to any substantive risks that could be greater than negligible (discussed in depth in sections 2.4.1-2.4.3; this chapter). One risk scenario was identified as posing substantive risk which warranted further assessment (characterised in Section 3; this chapter).

Table 4 Summary of hypothetical risk scenarios from dealings with GM vaccine

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
1	GM ILTV vaccine	Exposure of people conducting the dealings to the GMO via aerosols, fomites, contact with mucous membranes, needle stick: <u>During:</u> (a) preparation and administration of the GMO; (b) handling and transport of chickens vaccinated with the GMO; (c) unintentional spills; and (d) transport, storage or disposal of the GMO and waste	Disease in people	No	• Vaccination would be conducted by trained workers supervised by a registered veterinarian or qualified person. • Commercial poultry industries follow strict biosecurity procedures. • Transport of live chickens are regulated by state and territories in accordance with national standards and guidelines. • Storage and disposal of carcasses and other contaminated farm waste should follow local council and state requirements.

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		associated with GMO. ⬇ Infection of people with the GMO.			• There is no GMO detected in dust samples. • Other ILTV vaccines have a history of safe use with no adverse effects in people from direct exposure. • ILTV has a very narrow host range, is not a human pathogen and is not expected to cause disease, toxicity or allergenicity in people.
2	GM ILTV vaccine	Exposure of other people to the GMO via the consumption of meat, eggs or egg products. ⬇ Infection of people with the GMO.	Disease in people	No	• Chicken sold for human consumption lack the internal organs, gastrointestinal tract and head, which are the sites of infection of the GMO • Cooking will destroy the GMO. • Food products must adhere to the Food Standards Code. • Other ILTV vaccines have a history of safe use with no adverse effects in people indirectly (via consumption of meat/eggs).
3	GM ILTV vaccine	Exposure of other susceptible birds to the GMO via: (a) transmission from chicken farms; (b) transport of chicken and eggs; and (c) insect vectors. ⬇ Infection of other susceptible birds. ⬇ Exposed birds become infected ⬇ Disease in susceptible birds; or	Decreased numbers of susceptible birds or Increased numbers of feral/pest birds	No	• Exposure is minimised due to strict biosecurity procedures. • Measures are in place to control insect vectors (e.g. beetles). • Measures are in place to minimise wild birds accessing sheds, farms and water tanks in commercial poultry farms. • Local council and state requirements impose conditions to prevent contamination of water sources. • GMO is unable to maintain a stable presence in the

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		vaccination of feral/pest birds			environment for long periods. • Transmission of GMO to other chickens is rare. • GMO is unlikely to cause disease in native birds, including wild quails.
4	GMO	Vaccination of poultry farm chicken with the GMO. ⬇ Infection of cells by GMO. ⬇ Transduced cells co-infected with circulating ILTV strain. ⬇ Homologous recombination with ILTV (field, vaccine or WT strains). ⬇ (a) Reversion of GMO to WT strain; or (b) Generation of novel recombinant ILTV. ⬇ Infection of chickens and/or other susceptible avian species.	Disease in chickens or other susceptible species	Yes	• ILTV vaccines have a known history to recombine with WT strains resulting in novel strains that could infect and cause disease in susceptible avian species. • See Section 3 for risk characterisation

2.4.1 Risk scenario 1

Risk source	GM ILTV vaccine
Causal pathway	Exposure of people conducting the dealings to the GMO via aerosols, fomites, contact with mucous membranes, needle stick: <u>During:</u> (a) preparation and administration of the GMO; (b) handling and transporting of chickens vaccinated with the GMO; (c) unintentional spills; and (d) transport, storage or disposal of the GMO and waste associated with GMO. ⬇ Infection of people with the GMO.
Potential harm	Disease in people.

Risk source

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

Causal Pathway

195. People conducting the dealings could be exposed to the GMO in several ways. The GMO could be transmitted directly to mucous membranes via aerosol droplets generated during an unintentional spill of the GMO or during the preparation and administration of the GMO. Transmission could also occur through a needle stick injury if manual *in ovo* administration is used. People handling and transporting chickens vaccinated with the GMO or waste containing the GMO could be exposed to the GMO via contact (e.g. hands, trucks and containers used for transport). This exposure could potentially result in infection with the GMO that could lead to disease.

Exposure during preparation and administration of the GMO

196. As discussed in Chapter 1, Section 2.1, the GMO would be supplied as a freeze-dried vaccine in glass vials, which would need to be reconstituted prior to use. There is the potential for exposure of people involved in the administration of the GMO by aerosol formation during preparation and administration; from breakage/spillage of GMO onto surfaces during preparation and administration; or via needle stick injury (*in ovo* administration).

197. The GM vaccine would be prepared and administered by farm personnel under the direction of a veterinarian via eye drops, drinking water, coarse spraying and *in ovo* vaccination.

198. Administration via drinking water or via coarse spraying could potentially lead to exposure of people administering the vaccine via aerosol or spills. It is plausible that there would be residual GMO on the feathers of the chickens from coarse spraying or from contact with drinking water. Both these administration methods would be carried out in sheds, limiting the spread of the GMO. Farm personnel would need to comply with strict biosecurity procedures (e.g. use of clean protective clothing and boots; disinfection of hands and boots before and after entry; proper water and waste management) as described in Chapter 1, Section 5.4-5.7 to avoid exposure of people to the GMO while administering the vaccine.

199. *In ovo*, vaccinations are typically done via an automated machine in a commercial setting and the likelihood that people could get exposed to the GMO from this mode of administration is highly unlikely. The applicant has stated that the *in ovo* administration will be automated so no needlestick injuries could occur.

200. Based on the current registration for approved live ILTV vaccines, the APVMA registration of veterinary vaccines would include a label indicating the dosage; method of administration; precautions; personal protective equipment (PPE) requirements; and instructions relating to first aid, storage, and disposal of the GMO. The current APVMA permit for the field trial with the GMO includes safety information for handling the GMO (e.g. eye protection and masks) to ensure that exposure through the mucous membranes (eyes and airways) are minimised. Compliance with these behavioural practices at poultry farms would reduce the likelihood of unintended exposure of people to the GMO.

201. The existing work practices mentioned above would minimise the potential exposure of people to the GMOs during preparation and administration of the vaccine.

Exposure during handling and transport of the chickens inoculated with the GMO

202. As mentioned in Chapter 1, Section 3.5.2, ILTV is mainly transmitted by direct contact with respiratory and conjunctival exudates from infected chickens. Therefore, personnel in poultry farms may also be exposed to the GMO when handling chickens that have been vaccinated with the GMO or via materials or surfaces contaminated with the GMO during transport of vaccinated chickens through hand to mouth/eye transmission. As mentioned above, poultry personnel must adhere to strict biosecurity procedures (Chapter 1, Section 5.5), which includes proper protective clothing and footwear; sanitising hands; and exclusion for the day if they had contact with poultry or other birds (unless it is an emergency and they have a shower and change of clothes). This would limit the exposure of people to the GMO.

203. While some studies have reported the presence of ILTV DNA in poultry dust, no viable ILTV was recovered from ILTV PCR positive poultry dust. Furthermore, data has shown that ILTV PCR positive dust were unable to infect chickens when directly inoculated into the eyes of naïve chickens.

204. The transport of chickens to harvesting farms or layer farms are regulated by the state and territories in accordance with the *Australian Animal Welfare Standards and Guidelines – Land Transport of Livestock.* This would include the proper handling of the livestock and cleaning procedures for the vehicles transporting chickens.

205. Adherence to the biosecurity procedures and the *Australian Animal Welfare Standards and Guidelines – Land Transport of Livestock* would limit the exposure of the GMO to people handling the GMO.

Exposure via unintentional spill

206. If the GM vaccine was unintentionally/accidentally spilled or lost during transport or storage, this could result in exposure to people transporting or storing the GMO via the generation of aerosols through the mucous membrane and potential infection.

207. As described in Chapter 1, Section 2.1, the GMO would be packaged in glass-vials and subsequently packaged into a secondary containment containing dry ice and a cardboard box prior to transport for distribution. This would lower the likelihood of unintended dispersal of the GMOs.

208. The packaged final product will be stored in freezers in a distribution centre prior to transportation to farms. The current APVMA registered ILTV vaccines and the APVMA permit for field trials with the GM vaccine include storage instructions (in freezers) and to transport the vials in an insulated box with ice from the main storage area to areas of use around the farm before reconstitution.

209. The transport and storage procedures discussed above would meet the containment requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, which ensures that the GM vaccine would be properly contained for transport and storage. This would mitigate exposure due to spills of the GMO during these dealings.

Exposure during disposal of the GMO and waste contaminated with the GMO

210. Individuals may be inadvertently exposed to GMOs while disposing of used, expired, or unused vials of the GM vaccine. In addition, people could also possibly be exposed to the GMO during the disposal of waste contaminated with the GMO (e.g. poultry litter or dead chicken carcasses or water run-off).

211. As mentioned in Chapter 1, Section 2.1, the applicant has stated that all residual vaccine and associated waste which has come into contact with the GM vaccine (such as syringes, vials and eye droppers) would be discarded into solutions containing appropriate disinfectant (e.g. bleach) prior to disposal. As mentioned in Chapter 1, Section 3.5.6, ILTV is an enveloped virus that is sensitive to organic solvents and oxidising agents. These compounds are typically found in commercial disinfectants and would be sufficient to disinfect the GMO. Current registered ILTV vaccines have disposal instructions on their labels to dispose the vial/container appropriately.

212. The disposal of carcasses and litter generated from poultry farming would need to meet the requirements of the EPA from the various state and territories (Chapter 1, Section 5.5), which would take into account the possibility of water waste management, transmission to other people and the environmental impact. In addition, all waste and litter would also need to follow industry biosecurity standards, which all aim to minimise the impact of the waste on the environment.

213. No transmission of ILTV was observed via the excreta, blood or plasma of chickens.

214. The disposal and decontamination procedures discussed above would minimise the likelihood of exposure of people that could be associated with conducting these dealings with the GMOs.

Potential harm

215. As mentioned in Chapter 1, Section 3.5.1, ILTV has a very narrow host range and is not known to cause disease in humans. ILTV occurs naturally in the environment, and live attenuated ILTV vaccines are widely used in poultry, so people working in the poultry industry are currently exposed with no reports of disease, infection (clinical or subclinical), toxicity or allergic reactions. Similarly, there is no indication that the GM vaccine lacking one gene compared to ILTV would cause disease, infection (clinical or subclinical), toxicity or allergic reactions.

Conclusion

216. The potential of exposure of the GMO to people conducting the dealings leading to disease is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GM ILTV vaccine
Causal pathway	Exposure of other people to the GMO via the consumption of meat, eggs or egg products. ⬇ Infection of people with the GMO.
Potential harm	Disease in people.

Risk Source

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

Causal Pathway

218. Other people not involved with the dealings could potentially be exposed to the GMO via the consumption of meat, eggs or egg products.

219. It is proposed that chickens or eggs from chickens vaccinated with the GMO would enter the human food supply. Therefore, people may be exposed to the GMO, or to material from the GMO, when preparing or consuming meat or eggs from GMO-inoculated chickens or eggs (*in ovo* administration).

220. There are three registered ILTV live-attenuated vaccines in Australia. The majority of chicken meat sold for human consumption lacks the internal organs, gastrointestinal tract and the head where the GMO may be present. Even if present in trace amounts, the GMO is unlikely to survive the cooking process. In addition, any poultry products would need to adhere to the Primary Production and Processing (PPP) Standard for Poultry Meat (Standard 4.2.2) and PPP Standard for Eggs and Egg Products (Standard 4.2.5). These standards require poultry growers to identify and control food safety standards associated with poultry growing, processing, egg safety hazards and traceability of eggs to ensure a reduction in foodborne illnesses.

221. *In ovo* vaccination would only occur in eggs destined to hatch to produce layer or broiler chicken and not for eggs entering the food chain. The main reason for *in ovo* vaccination is to protect chicken from ILTV before they even hatch. Therefore, it is very unlikely that eggs that have been vaccinated with the GM vaccine will enter the food supply chain.

222. The GMO is not detected 7 dpv. Chickens are not usually processed for meat 7 days after vaccination as it is not typical to vaccinate a flock so close to processing for economic reasons. ILTV is also not known to be transmitted from chickens to eggs or surfaces of eggs.

223. Therefore, based on the reasons above, it is very unlikely that people consuming meat or eggs will be exposed to ILTV leading to disease.

Potential harm

224. As mentioned in Chapter 1, Section 3.5.1, ILTV has a very narrow host range and is not known to cause disease in humans. ILTV occurs naturally in the environment, and live attenuated ILTV vaccines are widely used in poultry, so people consuming meat or eggs could be currently exposed with no reports of disease, infection (clinical or subclinical), toxicity or allergic reactions. Similarly, there is no indication that the GM vaccine lacking one gene compared to ILTV would cause disease, infection (clinical or subclinical), toxicity or allergic reactions.

Conclusion

225. The potential of exposure of the GMO from consumption of meat, eggs or egg products resulting in disease in people is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.4.3 Risk scenario 3

Risk source	GM ILTV vaccine
Causal pathway	Exposure of other susceptible birds to the GMO via: (a) transmission from chicken farms; (b) transport of chicken and eggs; and (c) insect vectors. ⬇ Infection of other susceptible birds (e.g. other commercial poultry species; wild, feral, pest, native or pet/household birds). ⬇ Exposed birds become infected ↙ Disease in susceptible birds ↘ vaccination of feral birds
Potential harm	Decreased numbers of susceptible birds Or Increased numbers of feral/pest birds

Risk Source

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

Causal Pathway

227. Other susceptible birds (e.g. other commercial poultry species; wild, feral, pest, native or pet/household birds) may be exposed to the GMO via transmission from chicken farms; during the transportation of chickens and eggs; from the disposal of waste; from unintentional spills or via insect vectors.

Exposure to other susceptible birds through transmission from farms, waste and unintentional spills

228. When administered via eye drops, residual GMO could be present in the eyes of vaccinated chickens. Residual GMO could also be present in the feathers of vaccinated chickens from coarse spraying or when they come into contact with drinking water containing the GMO. There is a likelihood of low shedding of the GMO post-vaccination and from latent reactivation of the GMO when birds are stressed. Therefore, the GMO could potentially be transmitted to other susceptible birds outside the poultry farm from close contact with the residual GMO present in the eyes or feathers or droppings from GMO vaccinated chickens.

229. However, as discussed in Risk Scenario 1, the administration of the GMO is usually carried out in the shed, and typically automated (for *in ovo* inoculation), limiting the spread of the GMO. There is a likelihood that free range chickens could encounter other susceptible birds as they are allowed

outside the sheds. However, as mentioned in Chapter 1, Section 5.5.3, measures must be in place to discourage wild birds accessing the farm, and the number of susceptible wild birds near poultry farms would be expected to be low. Compliance with these biosecurity guidelines would minimise the exposure of other susceptible birds to the chickens vaccinated with the GMO. Transmission studies suggest minimal shedding of the GMO following vaccination (Chapter 1, Section 4.3.2) and although studies suggests potential latency of the GMO, it is unlikely that other susceptible birds would be exposed to equivalent or higher dose of the recommended administration dose of the GMO.

230. Other susceptible birds outside the poultry farms could be exposed to the GMO indirectly from contact with someone who has been in contact with the GMO while working in the poultry farm. This could occur via the potential exposure of susceptible birds to contaminated PPE during vaccine administration and handling of the chickens. However, as mentioned in Chapter 1, Section 5.5, poultry farms personnel would need to comply with biosecurity procedures to minimise any transmission outside the poultry farms or between sheds. This includes having change areas away from shed with clean protective clothing and boots; footbaths containing disinfectants at entry points; exclusion of pet birds and other non-commercial avian species from farms; measures to minimise the congregation of other wild birds in the facility; exclusion from farm areas if they have contact with other birds (e.g. pet birds) for the day; and a shed cleanout once birds are harvested before a new flock is introduced. Therefore, adherence to these biosecurity procedures would mean that the exposure of other susceptible birds outside the poultry farm from contaminated PPE is highly unlikely.

231. Other commercial poultry farms are present in Australia (e.g. turkey, pheasant and Japanese quail). Although unlikely, there is a potential that production of more than one commercially produced avian species be carried out in the same farm. Therefore, there is a possibility that the GMO could be transmitted to other sheds within the farm from workers or shared equipment. However, all commercial poultry farms follow strict biosecurity arrangements. As mentioned in Chapter 1, Section 5.5.2, if more than one commercially produced avian species is kept in the production area, they are to be managed separately with suitable biosecurity arrangements and shared equipment should be cleaned and disinfected between uses. Adherence to these biosecurity guidelines would limit the potential spread of the GMO between different sheds and farms.

232. Other susceptible birds could be exposed to the GMO via contact with waste (e.g. carcasses, litter, water run offs) from poultry farms. However, as mentioned in Chapter 1, Section 5.7, poultry farms have measures in place to minimise the gathering of wild animals accessing the waste areas; and avoid the contamination of surface waters, stormwater drains, waterways, catchment and ground waters. In addition, as mentioned in Risk Scenario 1, the disposal or carcasses and waste generated would need to meet the EPA requirements from various state and territories. These management procedures would limit the exposure of other susceptible birds to the waste generated in poultry farms.

233. ILTV is predominantly transmitted via direct contact. Airborne transmission could occur but at a much lower rate in ILTV vaccine strains as demonstrated in laboratory studies (Chapter 1, Section 3.5.1). Airborne transmission to other susceptible birds could potentially occur during the transport of vaccinated chickens from the farm, but this would involve generation of aerosol from chickens sneezing. Field trials with the GMP grade of the GM vaccine showed that transmission of the GMO from vaccinated chickens to unvaccinated chickens within the same shed is rare. In contrast, earlier studies with an experimental grade of the GM vaccine in chickens housed in isolators showed that the GM vaccine can transmit to other naïve chickens at the same rate as the WT parent strain.

234. The GMO could be released into the environment through a spill during transport, storage or disposal where susceptible birds could be exposed to the GMO. This could result in exposure of these birds to the GMO. As discussed in Risk Scenario 1, there are a range of measures in place that would

reduce the chances of GMO being released into the environment. In addition, ILTV has been shown to be inactivated by exposure to ultraviolet light for 60 seconds minimising the persistence of the virus in the environment.

235. In the unlikely event that the GMO is released into sewage water, it will be markedly diluted due to the small quantity of GMO present in a large volume of liquid waste or water. Therefore, it is highly unlikely that infection of birds could occur following exposure to an environmental source.

Exposure via transport of chickens or eggs

236. Transmission of the GMO could also occur if susceptible birds encounter trucks carrying vaccinated chickens/eggs. Chickens are transported for processing at 7-8 weeks of age (broilers) or to laying quarters at 16 - 18 weeks of age (layers). It is unlikely that farmers would vaccinate broilers close to the age of transport for economic reasons. In addition, chickens are assessed to determine if they are healthy and suitable for transport minimising any potentially active infection of chickens that could lead to possible shedding. ILTV has also not been shown to be transmitted to eggs or on eggshells. As mentioned in Chapter 1, Section 3.5.3 and 4.3.2, ILTV could potentially form a latent infection and that reactivation could potentially occur when birds are stressed during transport. The *Australian Animal Welfare Standards and Guidelines – Land Transport of Livestock*, includes various requirements to ensure that welfare of poultry and minimise stress on the livestock during transport. It would also be unlikely that other susceptible birds would be close enough to trucks carrying vaccinated chickens to be exposed to the GMO.

237. Other susceptible birds could also be exposed to the GMO via indirect contact with trucks contaminated with the GMO during transport. Proper cleaning procedures of transport crates/containers and transport trucks between journeys are included in the *Australian Animal Welfare Standards and Guidelines – Land Transport of Livestock*. This would minimise the contact of susceptible birds with surfaces that could have been potentially contaminated with the GMO during transport of livestock.

238. Overall, the exposure of other susceptible birds to the GMO via the transport of vaccinated chickens/eggs is highly unlikely.

Exposure via consumption of darkling beetles

239. As mentioned in Chapter 1, Section 3.5.1, ILTV has been detected in darkling beetles which are known pests in the poultry industry. Susceptible wild birds could potentially feed on these pests and get exposed to ILTV. However, it is not known if ILTV replicates in darkling beetles and whether ILTV could transmit in this manner. Current biosecurity measures require all broiler farms to control and manage vermin or pests at the farm, and to restrict access of wild birds to the production area including sheds or housing, water, and feed. Thus, the opportunity for exposure of susceptible birds to the GMO via this pathway is reduced.

Potential harm

240. If susceptible birds are exposed to the GMO, they could potentially develop mild or severe forms of ILTV disease. As mentioned in Chapter 1, Section 4.3, the experimental grade of the GMO resulted in a range of mortality rates in chickens (0% - 12.5%), whereas the GMP grade product did not cause any mortality even at 10 times the dose or after multiple doses (3 times, a week apart). As mentioned in Chapter 1, Section 4.3.1, the GMO is also less pathogenic when compared to the parent CSW-1 strain that was isolated in Australia.

241. As described in Chapter 1, Section 3.5.1, ILTV could potentially infect other birds in the *Phasianidae* family (e.g. commercial poultry species such as turkeys, quails and pheasants; or wild/feral species such as pheasants and peafowls; or native species such quails). It is unknown whether these birds would be susceptible to the GMO. They could potentially develop the same symptoms as chickens exposed to the GMO. Therefore, exposure of these bird species to the GMO

could potentially result in illness or death and decrease in populations, resulting in a decrease in commercial production, decrease in wild/feral birds or reduced numbers of native quail. However, the GMO has been shown to be less pathogenic than the parent strain of the virus, which is present in Australia, and the host immune response would likely clear the GMO.

242. Alternatively, as the GMO was shown to offer protection to vaccinated chickens from ILTV infection, exposure of wild/feral birds (e.g. pheasant and peafowl) could also potentially result in immunity towards ILTV. This could inadvertently result in increased numbers of wild pheasant and peafowl. However, for this scenario to lead to harm to the environment, a large number of wild pheasant and peafowl birds would have to become infected with the GMO, and ILTV would need to be an important factor limiting the populations. As mentioned in Chapter 1, Section 5.10, peafowls and pheasants are introduced species, not commonly found. The likelihood that these species of birds will be found in large numbers near commercial poultry farms is very low. Reported outbreaks of ILTV is also not common, therefore the likelihood that ILTV is an important limiting factor of pheasant and peafowl populations is very unlikely.

243. As mentioned in Chapter 1, Section 5.10, there are native quail species in Australia that belong to the *Phasianidae* family. In the unlikely event that these quail species are exposed to the GMO, quail species are found to be resistant to ILTV infection (Chapter 1, Section 3.5.1). Therefore, it is very unlikely that exposure of the GMO to these species would lead to disease, death or vaccination of the native quail species.

244. In the unlikely event that other types of commercial poultry (e.g. turkey, pheasant, Japanese quail) are exposed to the GMO, it could potentially result in some mortality in the flock. However, as the GMO is attenuated and a vaccine strain, it is more likely that the commercial poultry flocks exposed to the GMO would be indirectly vaccinated against ILTV.

Conclusion

245. The potential of indirect exposure of the GMO via transmission from chicken farms; during the transportation of chickens and eggs; or insect vectors resulting in decreased numbers of other commercial poultry flocks or other susceptible bird species due to disease causing death, or increased number of feral/pest birds that have an adverse impact on other species is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Section 3 Risk characterisation

246. Four risk scenarios were postulated and evaluated, as summarised in Table 4. The fourth risk scenario was identified as posing a substantive risk which warrants further assessment. This section provides more detail on the evaluation of this scenario.

3.1 Risk Scenario 4

Risk source	GM ILTV vaccine
Causal pathway	Vaccination of poultry farm chicken with the GMO ⬇ Infection of cells by GMO ⬇ Transduced cells co-infected with another ILTV strain ⬇ Homologous recombination with ILTV (field, vaccine or WT strains) ⬇ (a) Reversion of GMO to WT strain; or (b) Generation of novel recombinant ILTV ⬇ Infection of chickens and/or other susceptible avian species
Potential harm	Disease in chickens and/or other susceptible species

Risk source

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

Causal Pathway and likelihood assessment

248. The probability of recombination occurring in viruses is dependent on co-circulation of different viruses in the same geographical area, genetic similarity between the viruses, rate of co-infection of a host with both viruses and viral population size within the infected host. For recombination between a circulating strain of ILTV and the GMO to occur, both strains would need to be present and replicating in the same cell at the same time. This could occur if the chickens vaccinated with the GMO are exposed to another strain of ILTV or if chickens infected with ILTV are vaccinated with the GMO or two different vaccinations are administered to the same bird.

249. The GMO could also form a latent infection (Chapter 1, Section 4.3.1) in vaccinated chickens, whereby the GMO could be reactivated when the birds are stressed. Chickens with reactivated GMO could potentially be exposed to an ILTV infection, or to a second vaccine strain resulting in two strains being present at the same time. However, there are animal welfare guidelines and legislation in place that are regulated by states and territories to ensure that the stress on chickens is minimised during poultry farming or during transport. In addition, as mentioned in Risk Scenarios 1 and 3, strict biosecurity procedures are in place to ensure that any disease is not transmitted into and out of the shed/poultry farm.

250. In addition to factors in Risk Scenarios 1 and 3, sheds are also decontaminated and tested after birds are harvested to ensure that potential disease agents are removed to prevent the presence of ILTV in the shed when a new flock is introduced. This would reduce the likelihood of a new flock

being infected with ILTV before being vaccinated. Vaccination of chickens with the GMO should also generate immunity towards a circulating strain, minimising the window in which chickens would be infected with the virus.

251. As discussed in Chapter 1, Section 3.4, bioinformatic studies have postulated that new strains of ILTV have emerged from the recombination between multiple vaccines strains; and vaccine and circulating strains of ILTV.

252. If recombination does occur, it could result in the reversion of the GMO to the WT phenotype or generate a novel recombinant of ILTV, which will be discussed below. The WT or novel strain(s) of ILTV could potentially cause disease in chickens or other susceptible avian species.

Recombination or reversion to parent ILTV strain

253. As described in Chapter 1, ILTV is endemic in Australia and the GMO is generated by deleting the gG protein from a naturally occurring ILTV strain in Australia (CSW-1). Therefore, it is possible that the GMO could recombine with the parent CSW-1 ILTV resulting in the reversion of the GMO to the parent CSW-1 ILTV. Historically, the majority of the ILTV outbreaks in Australia are from Class 2 and 8 (2007-2009), and more recently Class 9 (2009-2015). During these periods, CSW-1 (Class 4) was not identified as the cause of any outbreaks in Australia. It is not known whether CSW-1 is still currently circulating in Australia, but based on historical data, it has not been reported to cause outbreaks from 2007-2015 and therefore, homologous recombination between the GMO and CSW1, resulting in the reversion to wild type is unlikely.

254. It was also shown that live attenuated vaccines are capable of reverting to WT strains after bird-to-bird passaging and could potentially result in a more virulent strain. However, studies with the GMO have shown that multiple passages in birds were not successful as the virus isolated from the trachea after the initial inoculation was unable to infect subsequent hosts. It is important to note that the three current live-attenuated vaccines have been attenuated via serial passaging in embryonated hen eggs, which may have resulted in random point mutation(s) that conferred the attenuated phenotype. In contrast, the GMO was made by a partial deletion of the gG gene from ILTV. The gene deletion would have an increased genetic and phenotypic stability in the GM viral genome compared to a point mutation, which is more capable of reversion to the wild-type sequence, especially if the virus has a high mutation rate (Hanley, 2011; Bull, 2015). Any recombinant carrying the gG gene deletion is expected to retain the associated attenuated phenotype of the GMO. Nevertheless, despite the seemingly low likelihood of the recovery of a viral gene from deletion in laboratory experiments, it is still unknown if what is observed in tissue culture could reproduced in an actual host organism (Bull, 2015; Jimenez-Guardeno et al., 2015).

255. In summary, the reversion of the GMO to the parent CSW-1 strain wild type via co-infection of CSW-1 or serial passaging is **highly unlikely**.

Generation of novel recombinant ILTV

256. As mentioned in Chapter 1, Section 3.4, recombination between strains of ILTV is possible and could potentially result in novel strains of ILTV. No recombination events were detected when chickens were inoculated with the GMO and V1-99 (a Class 4 field strain) or the Serva vaccine. However, as mentioned in Chapter 1, Section 4.3.3, recombination events were detected between V1-99 and CSW-1 (parent organism); and Serva and A20. As recombination of ILTV strains has been previously shown to occur, there is a likelihood that the GMO could recombine with other ILTV strains.

257. For recombination to occur, both the GMO and the other strain of ILTV must be present and replicating in the same cell at the same time. As mentioned in Chapter 1, Section 4.3.4, peer reviewed studies have shown that the GM vaccine confers protection to chickens against the current circulating ILTV strain in Australia (Class 9), thus making it unlikely that another ILTV strain would be replicating in the same cell. If a novel recombinant arises, it needs to be transmitted to other

chickens to establish and maintain its presence. It is not known how long a novel ILTV strain would need to recombine, infect, and establish itself as a new strain. If recombination occurs and results in a novel ILTV strain, broiler chickens are only kept for about 7-8 weeks before harvesting, limiting the potential of transmission of the new recombinant strain. Layer chickens on the other hand are kept for longer (20 - 78 weeks) but have shown to have a better targeted immune response, which probably contributes to their longer lifespan (Koenen et al., 2002). Although no experimental data is available, it is likely that vaccinated layer chickens would generate a better protective immunity against ILTV compared to broilers, so would be less likely to be infected with a second strain of ILTV.

258. Current APVMA registered vaccines and the current APVMA field trial permit for the GMO includes precautions on their labels to only vaccinate healthy birds. The vaccination of chickens with the GMO would confer protective immunity against ILTV. Strict biosecurity procedures are also in place to limit the interaction of chickens in commercial poultry with other avian species that may harbour ILTV infection.

259. Recombination could also occur between multiple vaccine strains if multiple live ILTV vaccines were used in the same flock. This would require either multiple vaccinations close together to have the different strains in the same cells at the same time, or for reactivation of a latent vaccine strain. Given the short lifespan of broilers, multiple vaccination is more likely to happen in layers. Current APVMA registration of the three live attenuated ILTV vaccines recommends not to use ILTV vaccines originating from genetically distinct ILTV strains concurrently in a flock or on a site to limit this risk of recombination.

260. Therefore, based on the data available and adherence to biosecurity guidelines, the likelihood that both the GMO and other ILTV are present in the same host/cells for recombination to occur, leading to an established novel pathogenic ILTV strain is **highly unlikely**.

261. As mentioned in Chapter 1, Section 5.8, sequence analysis suggested a phylogenetically close relationship between ILTV and PsHV-1. However, there has not been any studies showing that these viruses could recombine. PsHV-1 is not reported in wild birds in Australia and not known to infect chickens. Therefore, it is **highly unlikely** that both ILTV and PsHV-1 would co-infect chickens or wild birds simultaneously resulting in the generation of novel recombinant strains that could cause disease.

Consequence assessment

Reversion to parent ILTV strain

262. If reversion were to occur by mutation in birds or recombination with the parent CSW-1, the GMO could regain its pathogenicity and would have similar characteristics as the parent CSW-1 ILTV. It could likely cause respiratory disease in susceptible species (e.g. chickens, turkeys and pheasants) as described in Chapter 1, Section 3.1. The CSW-1 strain is derived from an Australian field isolate from 1959, so is not novel to Australia and would have contributed to the genetic make-up of the current pool of circulating viruses. Therefore, the consequence of reversion of the GMO to the parent ILTV strain to susceptible species would be **marginal** (minimal or no increase in harm to desirable components of the environment).

Generation of novel recombinant ILTV

263. If recombination between the GMO and other ILTV strains were to occur, it could result in the generation of a novel strain of ILTV. In the unlikely event of a novel more virulent ILTV strain arising from recombination between the GMO and another ILTV strain within a farm, the opportunity for it to spread to other susceptible birds would be restricted by high level of biosecurity measures and notification requirements for ILTV disease. Therefore, the consequence of resulting in a novel ILTV ranges from **minor** (minor increase in damage to desirable components of the environment that is reversible and limited in time and space or numbers affected) to **intermediate** (significant increase in

damage to desirable components of the environment that is widespread but reversible or of limited severity).

Risk estimate

264. The risk estimate is based on a combination of the likelihood and consequence assessments, using the Risk Estimate Matrix (see Chapter 2, Section 1), as described in the Regulator's Risk Analysis Framework 2013.

265. The potential consequence of reversion of the GMO to WT in chickens/other avian birds is considered **marginal**, with a probability of **highly unlikely**. The overall risk is therefore estimated to be **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation).

266. The potential consequence of the generation of novel ILTV via homologous recombination in chicken/other avian species are considered **minor** (minor increase in damage to desirable components of the environment that is reversible and limited in time and space or numbers affected) to **intermediate** (significant increase in damage to desirable components of the environment that is widespread but reversible or of limited severity), with a probability of **highly unlikely**. The overall risk is therefore estimated to be **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation) to **low** (risk is of minimal concern but may invoke actions for mitigation beyond standard practices).

Section 4 Uncertainty

267. Uncertainty is an intrinsic part of risk analysis[6]. There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

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

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

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

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

270. DIR-154 listed several areas of uncertainty to be addressed for the commercial release of the GMO or to justify a reduction in limits and controls, which included:

- the degree of attenuation of the GMO under field trial conditions; and
- the ability of the GMO to establish an infection cycle and persist in the environment.

271. Although, the applicant has provided additional data in this application, the additional data did not fully resolve the uncertainty.

272. For DIR-193, additional uncertainty is noted in relation to several points, including:

- the potential of the GMO to recombine and generate novel pathogenic strains of ILTV;
- the potential of the GMO infecting and causing disease in other wild birds; and
- the length of the protection from ILTV conferred by the GM vaccine.

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

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

Section 5 Risk evaluation

275. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

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

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

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

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

279. In the context of the range of measures already in place, including the operating guidelines and requirements of the other regulatory agencies, and considering both the short and long term, one of these scenarios was identified as representing a substantive risk requiring further assessment.

280. The likelihood and consequences of the substantive risk was characterised (Chapter 2, Section 3), and the level of risk estimated using the Risk Estimate Matrix, as described in the *Regulator's Risk Analysis Framework 2013* (see Chapter 2, Section 1).

281. The risk due to recombination of GMO with other ILTV, with the potential for transmission of replication competent ILTV to other susceptible avian species resulting in disease was estimated as posing a negligible to low risk to the environment.

282. Control measures are likely to be imposed by the APVMA during the registration process (eye drop administration) and the permit application (for coarse spraying and *in ovo* administration) to manage those risks. Control measures for administration are currently imposed for administration via drinking water for trials under permit PER91758 (APVMA) and DIR-154. However, since the product is yet to be registered with the APVMA and is not yet approved under permit for coarse spraying and *in ovo* administration, additional measures to mitigate the identified risk are considered in Chapter 3.

Chapter 3 Risk management plan

Section 1 Background

283. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

284. 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.

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

286. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

287. The risk identification process led to identification of one substantive risk, which involves the GMO recombining with other ILTV strains in chickens, with the potential for transmission of the replication competent GMO to other susceptible birds resulting in disease. This risk was characterised in Chapter 2, Section 3.

288. Current APVMA registered ILTV vaccines have warning statements on their labels to ensure that:

- ILTV vaccines originating from distinct ILTV strains should not be used concurrently in a flock or at a site; and
- only healthy birds are vaccinated.

This is to manage the risk of recombination between ILTV strains.

289. As the GM vaccine is yet to be registered by the APVMA and future experiments involving different administration methods are yet to receive a permit, it is proposed to include licence conditions to prevent the concurrent administration of live ILTV vaccines, and to only vaccinate healthy chickens to manage the risk of recombination.

290. There is a possibility that the reactivation of ILTV from a previous ILTV infection or vaccination could result in recombination with the GM vaccine. However, chickens previously infected with ILTV or vaccinated against ILTV would likely generate an immune response towards the GMO. This would minimise the likelihood of both the GMO and reactivated virus being present concurrently. Therefore, the licence condition to manage the risk of recombination is limited to preventing the concurrent administration of live ILTV vaccines, and to only vaccinate chickens that do not have clinical signs of an ILTV infection.

Section 3 General risk management

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

- applicant suitability
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting structures; and
- access for the purpose of monitoring for compliance; and
- other modes of administration.

3.1 Applicant suitability

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

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

293. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

3.2 Testing methodology

295. If a licence were issued, Bioproperties Pty Ltd would be required to provide a method to the Regulator for the reliable detection of the GMO, and the presence of the introduced genetic materials in a recipient organism. This methodology would be required prior to conducting any dealings with the GMO.

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

296. If a licence were issued, any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Modes of administration

297. The applicant has proposed to include various modes of administration (eye drops, drinking water, coarse spraying and *in ovo*). The administration by eye drops and drinking water has already been approved for trial under DIR-154 and an APVMA permit, but not for commercial use. Coarse spraying and *in ovo* vaccination are not currently authorised by the OGTR or APVMA for trials or commercial use. The risks associated with all these methods of administration have been included in the risk assessment for DIR 193.

3.5 Reporting requirements

298. If issued, the licence would oblige the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings;
- any contraventions of the licence by persons covered by the licence;

- any unintended effects of the release.

299. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

300. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

301. If issued, the licence would also oblige the licence holder to notify the Regulator of the following authorisations by the APVMA:

- inclusion on the Public Chemicals Registration Information System (PubCRIS);
- any amendments to the registration; and
- approval and subsequent amendments of permit(s) for the administration of the GMO.

3.6 Monitoring for compliance

302. 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 the Regulator, inspectors or other person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

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

Section 4 Post release review

304. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

305. For the current application for a DIR licence, the Regulator is including conditions that require ongoing oversight in order to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through PRR activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

306. Any member of the public can report adverse experiences/effects resulting from a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the risk assessment of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

307. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

308. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

309. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

310. The characterisation of the risk scenarios discussed in Chapter 2 identified the risk of recombination as a risk that is greater than negligible. Therefore, it was considered a substantive risk that warranted further detailed assessment. Uncertainty is considered to be low. No other specific indicators of harm have been identified in this RARMP for application DIR 193. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

311. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

312. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s) or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the consultation RARMP

313. The risk assessment concludes that the proposed commercial release of this GM ILTV vaccine poses negligible risks to the health and safety of people and a negligible to low risk to the environment as a result of gene technology.

314. The risk management plan concludes that the identified negligible to low risks can be managed to protect the health and safety of people and the environment by imposing risk treatment measures. Licence conditions are proposed to prevent the concurrent administration of vaccine with different ILTV strains and restrict the vaccination to healthy birds.

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

    (a) unless defined otherwise, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

    (b) words importing a gender include every other gender;

    (c) words in the singular number include the plural and words in the plural number include the singular;

    (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no-one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;

    (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

    (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;

    (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

‘**Act**’ means the *Gene Technology Act 2000* (Cth) or the corresponding State legislation under which this licence is issued.

‘**Annual Report**’ means a written report provided to the Regulator by the end of September each year containing all the information required by this licence to be provided in the Annual Report.

‘**GM**’ means genetically modified.

‘**GMO**’ means the genetically modified organism that is the subject of the dealings authorised by this licence.

‘NLRD’ is a Notifiable low risk dealing. Dealings conducted as an NLRD must be assessed by an institutional biosafety committee (IBC) before commencement and must comply with the requirements of the Gene Technology Regulations 2001.

‘**OGTR**’ means the Office of the Gene Technology Regulator.

‘**Regulator**’ means the Gene Technology Regulator.

Section 2 Licence conditions and obligations

3. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMO are authorised during any period of suspension.

4. The licence holder is Bioproperties Pty Ltd.

5. Any person, including the licence holder, may conduct any authorised dealing(s) with the GMO.

6. The dealings authorised by this licence are:

    (a) conduct experiments with the GMO;

    (b) transport of the GMOs;

(c) disposal of the GMOs;

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

Note: Use of the GMO for veterinary purposes is not covered by the Gene Technology Act 2000 and therefore this licence is not required to authorise such use. The GMOs are also subject to regulation by other federal and state departments and agencies, including the Australian Pesticides and Veterinary Medicines Authority and the Department of Agriculture, Fisheries and Forestry. These other departments and agencies may impose further requirements for, or limitations on, the use of the GMO or these dealings.

7. This licence does not apply to dealings with the GMOs conducted as a Notifiable Low Risk Dealing (NLRD) or pursuant to another authorisation under the Act.

Note: Dealings conducted as an NLRD must be assessed by an Institutional Biosafety Committee (IBC) before commencement and must comply with the requirements of the Regulations

8. Dealings with the GMO may be conducted in all areas of Australia.

9. Dealings described in Condition 6(a) must not occur unless authorised by a permit or registration by the APVMA.

10. The licence holder must ensure that end users of the GMO are informed that the GMO:

(a) is not to be given concurrently with any other live ILTV vaccines; and

(b) is to be given to healthy chickens only.

11. The licence authorises dealings with the GMO described in **Attachment A**.

12. To the extent that the conditions of any prior licence authorising dealings with the GMOs are inconsistent with the conditions of this licence, the conditions of this licence will prevail.

2.1 Obligations of the Licence Holder

13. The licence holder must immediately notify the Regulator if any of its contact details change.

Note: Please address correspondence to OGTR.M&C@health.gov.au

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following conditions address ongoing suitability of the licence holder.

14. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.

15. The licence holder must:

(a) inform the Regulator immediately in writing, of:

i. any relevant conviction of the licence holder; and

ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; and

iii. any event or circumstances that would affect the capacity of the holder of this licence to meet the conditions in it; and

(b) provide any information related to the licence holder's ongoing suitability to hold a licence, if requested, within the stipulated timeframe.

16. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:

(a) the particular condition (including any variations of it); and

(b) the cancellation or suspension of the licence; and

(c) the surrender of the licence.

2.2 Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following condition requires that any new information that may affect the risk assessment is communicated to the Regulator.

17. The licence holder must inform the Regulator if the licence holder becomes aware of:

(a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

(b) any contraventions of the licence by a person covered by the licence; or

(c) any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

(a) the licence holder will be taken to have become aware of additional information of a kind mentioned in paragraph 17 if he or she was reckless as to whether such information existed; and

(b) the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in paragraph 17, if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.

Note: Contraventions of the licence may occur through the action or inaction of a person.

18. If the licence holder is required to inform the Regulator under condition 17, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made at the time of the incident via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours.

19. If at any time the Regulator requests the licence holder to collect and provide information about any matter to do with the progress of the dealings authorised by this licence, including but not confined to:

(a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 17(a);

(b) any contraventions of the licence by a person covered by the licence, whether or not the licence holder has provided information to the Regulator under condition 17(b);

(c) any unintended effects of the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 17(c);

(d) research, including by way of survey, to verify predictions of the risk assessment, or for any purpose related to risks to the health and safety of people, or to the environment;

(e) scientific literature and reports in respect of the GMO authorised by this licence, for a nominated period;

(f) details of any refusals of applications for licences or permits (however described) to deal with the GMO made pursuant to the regulatory laws of a foreign country;

and the request is reasonable, having regard to consistency with the Act and relevance to its purpose, then the licence holder must collect the information and provide it to the Regulator at a time and in the manner requested by the Regulator.

Note: The Regulator may invite the licence holder to make a submission on the reasonability of a request by the Regulator to collect and provide information relevant to the progress of the dealings with the GMO.

2.3 Obligations of persons covered by the licence

20. If a person is authorised by this licence to deal with the GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Section 3 Reporting and Documentation Requirements

3.1 Notification of authorisations by the Australian Pesticides and Veterinary Medicines Authority

21. If the GMOs are included on the Public Chemical Registration Information System (PubCRIS), the licence holder must notify the Regulator in writing and include information of how Condition 10 is met, within 14 days of registration.

22. The licence holder must notify the Regulator in writing of any subsequent amendments to the conditions of the PubCRIS registration involving the pattern of usage, handling, storage, transport or disposal of the GMOs, within 14 days of the change occurring.

23. The licence holder must notify the Regulator in writing within 14 days of obtaining a permit from the APVMA, including any amendments of current and future permits, and include information of how Condition 10 is met.

3.2 Annual Report

24. The licence holder must provide an Annual Report to the Regulator by the end of September each year covering the previous financial year. An Annual Report must include:

(a) information about any adverse impacts, unintended effects, or new information relating to risks, to human health and safety or the environment caused by the GMOs or material from the GMOs;

(b) information about the numbers of GM vaccine doses distributed to each State and Territory.

3.3 Testing methodology

25. At least 14 days prior to conducting any dealings with the GMO, the licence holder must provide to the Regulator a written methodology to reliably detect the GMO, or the presence of the genetic modifications described in **Attachment A** in a recipient organism or environmental sample. The detection method(s) must be capable of identifying, to the satisfaction of the Regulator, the genetic modification event described in **Attachment A**.

Note: Please address correspondence to OGTR.M&C@health.gov.au

ATTACHMENT A

DIR No: 193

Full Title: Commercial supply of a genetically modified vaccine against infectious laryngotracheitis virus in chickens

Organisation Details

Postal address: Bioproperties Pty Ltd
36 Charter Street
Ringwood VIC 3134

Phone No: (03) 9871 2000

Accreditation No: Accr 131

GMO Description

GMOs covered by this licence

The GM vaccine contains a live attenuated infectious laryngotracheitis virus (ILTV) CSW-1 strain. The GM vaccine was produced by the deletion of glycoprotein G from its genome.

Parent Organism

Common Name: Infectious laryngotracheitis virus (ILTV)

Scientific Name: *Gallid herpesvirus 1*

Modified traits

Category: Vaccine – attenuated

Description: ILTV has been genetically modified to reduce pathogenicity and virulence, for use as live attenuated vaccine.

Purpose of the dealings with the GMO

The purpose of the dealings is commercial supply of the GM vaccine against ILTV Australia-wide to provide protection against ILTV infection. In addition, the efficacy and safety of the GMO using different administration methods will be tested prior to commercial supply. Therefore, the permitted dealings under this licence are transport, storage and disposal of the GM vaccines and conduct experiments with the GMO.

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Appendix A: Summary of submissions

The Regulator received several submissions from prescribed experts, agencies and authorities[7] on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	No expertise in this area to be able to provide comments.	Noted.
2	No specialist scientific expert to make an assessment and no comments provided.	Noted.
3	No expertise to comment but proposed to discuss with state contact.	Noted. Other prescribed agencies and state and territories have been consulted on the proposal.
4	Issues raised: • concerned about the potential risk to the people that may be affected by the commercial release of the GM vaccine; • requested that residents surrounding the council area to be advised; • requested contact person to refer enquiries and complaints to; and • assumes state contact has been advised.	Noted. The potential risk regarding the commercial release of the GM vaccine has been addressed in the RARMP prepared by the Regulator. The RARMP will be sent out for consultation to the public, prescribed agencies, state and local government areas with contact details for responses. Prescribed agencies and state and territories have been consulted on the preparation of the RARMP.
5	Advised to consider the following in preparation of the RARMP: • recent research on replication ability, shedding transmission, recombination and potential exposure (via dust, faeces, litter, water, air and wind) of the GM vaccine or possible recombinants to native wild bird species; • include possibilities of air and wind-borne transmission of the virus and discuss management	The potential for viral replication, shedding, transmission, potential for recombination of the parent organism and GM vaccine have been discussed throughout Chapter 1 (Section 3.5 and Section 4.3) and Chapter 2 (Risk scenarios 1 to 4).

[7] Prescribed expects, agencies and authorities include GTTAC, State and Territory Governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	measures that would reduce transmission and exposure of native birds, including native quail species; • reversion and recombination in ILTV (field or vaccine strains) leading to persistence of ILTV in Australia and the emergence of novel strains that can cause disease in other chickens or native wildlife; and • assess the quality of the data provided with the application.	
6	Questions were raised on the: • persistence of the virus in the environment; • transmission of the virus to unvaccinated chickens; • efficacy of the GM vaccine compared to other ILTV vaccines; • potential for recombination between GM vaccine and field strains; and • stability of the GM vaccine.	Noted. The potential persistence, transmission, stability and potential for recombination of the parent organism have been discussed throughout Chapter 1 (Section 3.5 and Section 4.3) and Chapter 2 (Risk scenarios 1 to 4). The efficacy of the GM vaccine is outside of the scope of the Regulator's assessment and will be considered by the APVMA in their registration process.
7	No concerns with the application. Comments: • vaccine should be able to provide protection even though it is non-pathogenic; • unlikely GMO will enter the food chain and animal feed due to slaughtering, cooking and manufacturing processes; and • stability of the GM vaccine.	Noted. The efficacy of the GM vaccine is outside of the scope of the Regulator's assessment and will be considered by the APVMA in their registration process. The likelihood of harm from the GMO entering the food chain is addressed in Chapter 2 (Risk scenario 2). The stability of the GM vaccine is addressed in Chapter 1 (Section 4.3.3).
8	Department has advised to consider the following in preparation of the RARMP: • persistence of the GMO in the environment;	Noted. The potential persistence, transmission, stability

Submission	Summary of issues raised	Comment
	• transmission to unvaccinated chickens; • efficacy of GM vaccine compared to current registered vaccines; • recombination between GM vaccine and field strains of ILTV; and • genetic stability of the GM vaccine in field trials.	and potential for recombination of the parent organism have been discussed throughout Chapter 1 (Section 3.5 and Section 4.3) and Chapter 2 (Risk scenarios 1 to 4). The efficacy of the GM vaccine is outside of the scope of the Regulator's assessment and will be considered by the APVMA in their registration process.
9	**Draft recommendations** The committee agrees that the following should be included in the RARMP: • potential accidental exposure of humans and other organism to the GMO resulting in harm; • potential for complementation and recombination of the GMO and other ILTV; and • potential for GMO to be harmful to the environment.	Noted. The potential for accidental exposure, complementation and recombination of the GMO and potential for GMO to be harmful to the environment have been discussed throughout Chapter 1 (Section 3.5 and Section 4.3) and Chapter 2 (Risk scenarios 1 to 4).