



Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

**Risk Assessment and
Risk Management Plan for
DIR 098**

**Commercial release of a genetically modified live viral
vaccine to protect against Japanese encephalitis
(IMOJEV™)**

Applicant: Sanofi Pasteur Pty Ltd

August 2010

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in response to a licence application (DIR 098) from Sanofi Pasteur Pty Ltd (Sanofi) for a commercial release of a genetically modified (GM) vaccine.

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

The application

The Regulator received an application from Sanofi for a licence for dealings involving the intentional release of a live genetically modified (GM) viral vaccine for the prevention of Japanese Encephalitis.

The GM vaccine is based on the existing vaccine for Yellow fever in which two genes have been replaced by similar genes from *Japanese encephalitis virus*. It has been shown to protect vaccinated people against Japanese Encephalitis.

Sanofi proposed a commercial release of this vaccine in medical facilities throughout Australia. The vaccine is intended for people travelling to, or resident in, areas where the disease occurs and will be prescribed by registered medical practitioners and administered in medical facilities.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities that were consulted on the preparation of the RARMP and the consultation RARMP. The risk context for this assessment considered the dealings import, transport and disposal associated with the commercial release. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

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

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

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

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

The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm did not give rise to any identified risks that required further assessment.

Any risks of harm to the health and safety of people or the environment from the dealings associated with the proposed release, which are the import, transport and disposal of the GM vaccine, are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this commercial release **do not pose a significant risk** to either people or the environment.

Risk management plan

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

As none of the five risk scenarios characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to allow appropriate oversight of the ongoing release.

The imposed licence conditions ensure that there is appropriate oversight of the ongoing release and require Sanofi to inform the Regulator of any amendments to the conditions of the TGA registration involving the pattern of use, handling, storage, transport or disposal of the GMO.

Conclusions of the consultation RARMP

The risk assessment concluded that the dealings associated with this commercial release of the GM vaccine as a prescription medicine pose **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to allow appropriate oversight of the ongoing release.

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Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
ARTG	Australian Register of Therapeutic Goods
C	<i>flavivirus</i> capsid protein
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic Acid
DNIR	Dealings <u>Not</u> involving Intentional Release
DoHA	Australian Government Department of Health and Ageing
E	<i>flavivirus</i> envelope protein
ER	endoplasmic reticulum
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GMO	Genetically Modified Organism
GTECCC	Gene Technology Ethics and Community Consultative Committee
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal Gene Transfer
IC	Intracranial
IFN	Interferon
JE	Japanese encephalitis
JEV	<i>Japanese encephalitis virus</i>
ml	millilitre
MVEV	<i>Murray Valley encephalitis virus</i>
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NS	Non structural
OGTR	Office of the Gene Technology Regulator
OH&S	Occupational health and safety
ORF	Open reading frame
pfu	plaque forming units
prM	<i>flavivirus</i> pre-membrane protein
PRR	Post Release Review
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNA	Ribonucleic acid
SA 14-14-2	<i>Japanese encephalitis virus</i> vaccine strain SA 14-14-2
Sanofi	Sanofi Pasteur Pty Ltd
SC	subcutaneous
TGA	Therapeutic Goods Administration
USA	The United States of America
UTR	Untranslated Region
UV	Ultra Violet
WNV	<i>West Nile virus</i>
YEL-AND	Yellow fever vaccine-associated neurotropic disease

YEL-AVD	Yellow fever vaccine-associated viscerotropic disease
YF	Yellow fever
YF 17D	Yellow fever vaccine strain 17D
YFV	<i>Yellow fever virus</i>

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in response to a licence application (DIR 098) from Sanofi Pasteur Pty Ltd (Sanofi) for a commercial release of a genetically modified (GM) vaccine.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public².

The application

The Regulator received an application from Sanofi for a licence for dealings involving the intentional release of a live genetically modified (GM) viral vaccine for the prevention of Japanese Encephalitis.

The GM vaccine is based on *Yellow fever virus* vaccine strain YF 17D which has been modified to contain genes from *Japanese encephalitis virus* (JEV) vaccine strain JE SA14-14-2. Expression of these genes has been shown to elicit a protective immune response in vaccinated people.

Sanofi proposed a commercial release of this vaccine in medical facilities throughout Australia. The vaccine is intended for people travelling to, or resident in, areas where the disease occurs and will be prescribed by registered medical practitioners and administered in medical facilities.

Risk assessment

The risk assessment took into account information in the application, previous approvals, relevant scientific/technical knowledge and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities (included in Appendices B and C of the RARMP) as well as the public (included in Appendix D of the RARMP). The risk context for this assessment considered the dealings import, transport and disposal associated with the commercial release. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

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

Five risk scenarios were identified. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM virus; or

² More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

produce unintended changes in its characteristics. The opportunity for gene transfer to other organisms and its effects if this occurred was also assessed.

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

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

- long history of safe use of the parent vaccine viruses containing the same proteins or sequences encoded by the introduced genes with no evidence of harm to otherwise healthy people
- limited ability of the GM virus to replicate in humans and other animals
- limited ability of the GM virus to replicate in mosquito vectors
- limited ability and opportunity for the GM vaccine to transfer the introduced genes

Any risks of harm to the health and safety of people or the environment from the dealings associated with the proposed release, which are the import, transport and disposal of the GM vaccine, are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this commercial release **do not pose a significant risk** to either people or the environment.

Risk management plan

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

As none of the five risk scenarios characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. The Regulator's Risk Analysis Framework defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. Nonetheless, as part of the Regulator's oversight of licensed dealings involving the release of genetically modified organisms, the licence 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.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)³.

³ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* (OGTR 2009) available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

The TGA is responsible for assessing the quality, safety and efficacy of medicines and other therapeutic goods for use in Australia. The TGA has registered the GM vaccine for use in Australia, as a prescription medicine, for people over the age of 12 months.

Suitability of the applicant

The Regulator determined, at the commencement of the assessment process for this application, that Sanofi was suitable to hold a DIR licence under the requirements of section 58 of the Act. The Regulator is satisfied that Sanofi remains suitable as no relevant convictions have been recorded, and no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment.

Conclusions of the consultation RARMP

The risk assessment concluded that the dealings associated with this commercial release of the GM vaccine as a prescription medicine pose **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to allow appropriate oversight of the ongoing release.

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Chapter 1 Risk context

Section 1 Background

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

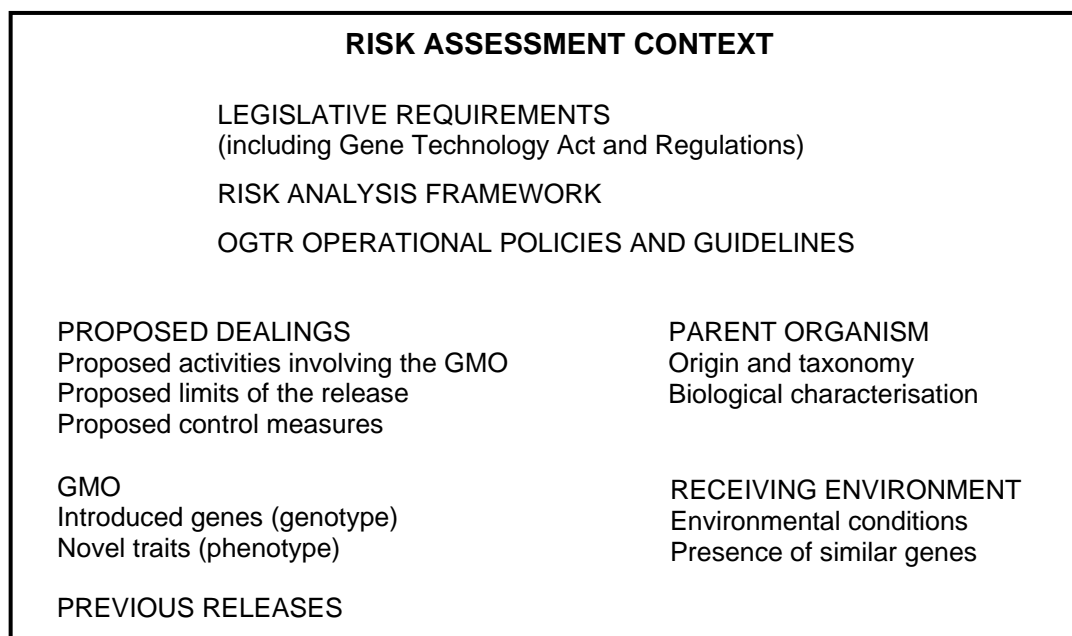


Figure 1 Components of the risk context considered during the preparation of the risk assessment

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* (the Act) and *Gene Technology Regulations 2001* (the Regulations) (Section 2) the Regulator's *Risk Analysis Framework* (OGTR 2009), and operational policies and guidelines (see <http://www.ogtr.gov.au>).

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

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

Section 2 The legislative requirements

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

5. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator was required to consult with prescribed experts, agencies and authorities to seek advice 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 or agencies prescribed in the Regulations, any local council that the Regulator considered appropriate⁴ and the Minister for Environment Protection, Heritage and the Arts. A summary of issues contained in submissions received is given in Appendix B. The Regulator has also sought advice from the Gene Technology Ethics and Community Consultative Committee (GTECCC) on the consultation process in relation to DIR 098.

6. Under Section 52 of the Act, the Regulator was required, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix C. Three submissions were received from the public and their consideration is summarised in Appendix D. The applicant also made a submission to the Regulator which was taken into account in finalising the RARMP.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period⁵ as specified in section 52(2)(d), see Chapter 2, Section 3. The Regulator considered that the dealings proposed do not pose a significant risk to either people or the environment.

Section 3 Scope and boundaries

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be registered on the Australian Register of Therapeutic Goods (ARTG). The Therapeutic Goods Administration (TGA) is responsible for administering the provisions of this legislation.

9. The TGA also regulates the labelling, handling, sale and supply of scheduled medicines through the *Standard for the Uniform Scheduling of Drugs and Poisons*⁶ (Poisons Standard 2009). These regulations are then enforced through state and territory poisons legislation which provides for licensing of people or organisations to handle scheduled medicines and poisons and also sets forth criminal penalties for improper labelling, handling, sale or supply of scheduled medicines (West Australia 1964; New South Wales 1966; Victoria 1981; Northern Territory 1983; South Australia 1984; Queensland 1996; South Australia 1996; Tasmania 2000b; Victoria 2006; Australian Capital Territory 2008).

10. Where a GMO is proposed to be a registered therapeutic, TGA has primary regulatory responsibility for quality, efficacy and patient safety; however authorisation is also required under gene technology legislation. The Regulator notes that the TGA has assessed risks to patients and will manage any risks identified. In order to avoid duplication of regulatory oversight the Regulator has assessed risks posed to other people who may be involved in the

⁴ In this instance, the Regulator decided to consult with all local councils in Australia.

⁵ In this instance, the Regulator allowed 8 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public

⁶ It should be noted that after 1 July 2010 this schedule will be known as the *Standard for the Uniform Scheduling of Medicines and Poisons* (Poisons Standard 2010)

dealings and to the environment. In the context of this RARMP risks to people receiving the vaccine has not been considered as part of the evaluation.

11. The Australian Government Department of Health and Ageing (DoHA), and the National Health and Medical Research Council (NHMRC) have issued the *National Immunization Handbook* (National Health and Medical Research Council 2008) which provides guidance on the procedures required for the safe administration of vaccines. This document provides Australian clinical guidelines on the safest and most effective use of vaccines. Additionally DoHA has published the *National vaccine storage guidelines: Strive for 5* (Department of Health and Ageing 2005) which provides guidance on the safe storage and handling of vaccines within Australia.

12. The disposal of clinical waste, unused/expired pharmaceuticals and other potentially biohazardous substances is regulated through state and territory waste management, occupational health and safety (OH&S) and environmental protection legislation (see for example Australian Capital Territory 1991a; New South Wales 1997; Queensland 2000; Victoria 2000; Tasmania 2000a; West Australia 2004; Northern Territory 2009; South Australia 2009) as enforced by local government, and would be carried out in accordance with the relevant Australian standard and industry codes of conduct (Standards Australia & Standards New Zealand 1998a; Standards Australia & Standards New Zealand 1998b; Biohazard Waste Industry Australia and New Zealand (BWI) 2007).

Section 4 The proposed dealings

13. Sanofi Pasteur Pty Ltd (Sanofi) propose to release a vaccine based on Yellow Fever (YF) vaccine strain 17D that has been genetically modified (GM) to contain genes from *Japanese encephalitis virus* (JEV) vaccine strain SA 14-14-2 as a prescription medicine. Previous clinical studies have shown that this GM vaccine elicits a protective immune response against JEV in vaccinated adults and children aged 12 months and over.

14. The purpose of the dealings is the ongoing commercial release of the GM vaccine known as IMOJEV™⁷.

15. The GM vaccine is intended for people travelling to, or residing in, areas where JEV is endemic and therefore Sanofi proposes the commercial release to occur in medical facilities throughout Australia. The imported GM vaccine would be distributed and sold through normal pharmaceutical distribution channels Australia-wide and may also be exported to New Zealand for subsequent distribution.

16. Sanofi also applied to the TGA to have IMOJEV™ included on the ARTG and listed as a Schedule 4 medicine (Prescription Only Medicine) according to the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009). IMOJEV™ would therefore, only be available on prescription by a registered medical practitioner.

17. Initially the applicant had proposed that supply be limited to persons over 18 years of age. However, the applicant has since applied to TGA and the Regulator that supply be permitted to persons over 12 months of age. TGA has advised that the vaccine has been registered for use in Australia by people over the age of 12 months.

18. As this is an ongoing commercial release, there are implications for long term considerations that would not necessarily be relevant to a limited and controlled release of short duration. These long term considerations are reflected in the risk assessment (Chapter 2) and risk management (Chapter 3) for the proposed release.

⁷ As IMOJEV™ has been registered by TGA, it is expected to be marketed as IMOJEV®.

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

- importing the GMO;
- transporting the GMO; and
- disposing of the GMO.

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

4.1 The proposed activities

20. The GM vaccine proposed for release would be imported from overseas manufacturing sites including Thailand and transported to central storage in Sydney, and distribution sites in Brisbane, Melbourne and Perth, before being transported to hospitals, pharmacies, general medical practices and travel clinics. Storage, handling and transport would be expected to take place in accordance with the *Australian Code of Good Wholesaling Practice For Therapeutic Goods For Human Use* (Therapeutic Goods Administration 1991) and also the *WHO Good distribution practices for pharmaceutical products* (World Health Organisation 2010). If IMOJEV™ is approved for marketing by the appropriate New Zealand authorities the GM vaccine may also be exported to New Zealand from the central storage site in Sydney.

21. Transport within Australia will be carried out through the use of commercial courier companies experienced in the transportation of pharmaceutical products, such as live vaccines, which require secure handling and the maintenance of a strict temperature regime (2°C to 8°C).

22. The applicant has proposed that the GM vaccine would be supplied as a lyophilised pellet or powder within a hermetically sealed glass vial. The vial of GM vaccine and a hermetically sealed glass vial of diluent solution would be placed in a protective tray along with a disposable syringe and two needles. The plastic tray would then be placed in a labelled carton for transport and handling purposes.

23. Once at the pharmacy or final destination, the GM vaccine would be stored in an outer package in a secure location with access limited to pharmacy and medical staff according to DoHA's *National vaccine storage guidelines: Strive for 5* (Department of Health and Ageing 2005) and the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009).

24. All of the inoculations would be conducted by trained medical staff and be undertaken at the medical or clinical facilities. All staff providing inoculations would be expected to follow all relevant guidelines including those in the *Australian Immunization Handbook* (National Health and Medical Research Council 2008), the *Infection control guidelines for the prevention of transmission of infectious diseases in the health care setting* (Department of Health and Ageing 2004), and the World Health Organisation universal precautions for the prevention of transmission of infectious agents in healthcare settings (World Health Organisation 2007).

25. Following administration, used vaccine syringes would be placed immediately into locked containers or sealed bags and destroyed following institutional procedures for the disposal of biohazardous material. Waste generated during vaccination would be discarded into appropriate biohazard containers and disposed of following institutional procedures for the disposal of biohazardous material in accordance with the requirements of the *Occupational Health and Safety Act 1991* (Commonwealth of Australia 1991) and related state and territory legislation. Unused vaccine would be returned to Sanofi's central storage depot in Australia or

disposed of at the pharmacy or medical practice following institutional procedures for the disposal of biohazardous material in accordance with the relevant state and territory legislation as discussed above.

26. Australian Product Information and Consumer Medicine Information documents have been submitted to the TGA for approval and will be made available to health care professionals and consumers respectively. The Product Information document will instruct health care professionals in the use and storage requirements for the GM vaccine. Similarly the Consumer Medicine Information document will inform the consumer about the GM vaccine, its use and method of administration, and other relevant safety information.

Section 5 The parent organisms

27. The GM vaccine is based on Yellow fever vaccine strain YF 17D which has been modified to contain genes from the Japanese encephalitis vaccine strain JE SA14-14-2. These highly attenuated vaccine strains were developed through artificial selection processes and do not exist naturally in the environment. As such, a discussion of the parent organisms carried out at the species level, rather than strain level, is required to inform the risk assessment.

5.1 Yellow fever virus and Japanese encephalitis virus taxonomy

28. The parent organisms *Yellow fever virus* (YFV) and *Japanese encephalitis virus* (JEV) are single stranded, positive sense RNA viruses of the Family *Flaviviridae* (ICTV 2009). The taxonomy of YFV and JEV are outlined in Table 1. The family *Flaviviridae* includes three genera, the *Flavivirus*, *Hepacivirus* and the *Pestivirus*. The genus *Flavivirus* contains highly pathogenic hemorrhagic fever viruses such as *Yellow fever virus* and *Dengue virus*, encephalitic viruses such as *Japanese encephalitis virus*, *Murray Valley encephalitis virus* (MVEV) and *West Nile virus* (WNV), and a number of less pathogenic viruses such as *Modoc virus*, *Kokobera virus* and *Kunjin virus*. The genus *Hepacivirus* contains the human pathogen *Hepatitis C virus* while the genus *Pestivirus* contains a number of animal pathogens including *Classical swine fever virus*, *Bovine viral diarrhea virus 1, 2 and 3* and *Border disease virus*.

Table 1 Taxonomy of *Yellow fever virus* and *Japanese encephalitis virus*

	<i>Yellow fever virus</i>	<i>Japanese encephalitis virus</i>
Family	<i>Flaviviridae</i>	<i>Flaviviridae</i>
Genus	<i>Flavivirus</i>	<i>Flavivirus</i>
Subgenus	<i>Yellow fever virus</i> group	<i>Japanese encephalitis virus</i> group
Species	<i>Yellow fever virus</i>	<i>Japanese encephalitis virus</i>
Strain	<i>Yellow fever virus strain 17D</i>	<i>Japanese encephalitis virus strain SA-14</i>

29. *Yellow fever virus* was the first member of the family to be characterised, and the name *Flaviviridae* is derived from the Latin word ‘flavi’ meaning yellow, which refers yellow skin colour (known as ‘jaundice’) typically observed in people with severe yellow fever. YFV and JEV are not generally considered endemic to Australia, although there have been seasonal outbreaks of JEV in outer islands of the Torres Strait. However, members of the JEV group endemic to Australia include MVE and *Alfy virus* (Burke & Monath 2001). The closely related *Kunjin virus*, and *Kokobera virus* group of viruses are also found in Australia (Nisbet et al. 2005).

5.2 Transmission and distribution

5.2.1 Yellow fever virus

30. *Yellow fever virus* is known as an arbovirus (arthropod borne virus) as transmission occurs via an insect vector. The primary vector for YFV is the mosquito *Aedes aegypti*, which requires a blood meal in order to reproduce. Following ingestion of a blood meal infected

with the virus, YFV is able to penetrate the gut and replicate systemically throughout the mosquito, a process which takes between ten and twenty one days. Infection of the salivary glands allows transmission to new vertebrate hosts through subsequent blood meals, while infection of reproductive tissues allows YFV to be passed on to offspring. Infected male progeny can also infect female mosquitoes during copulation. An infected mosquito will remain infectious for the entire period of its life, which in some instances can exceed three months. YFV infection is generally asymptomatic in mosquitoes and some non-human primates. YFV can also be transmitted by a number of other *Aedes spp.* and *Haemagogus spp.* mosquitoes. YFV has also been isolated from *Sabethes chloropterus*, *Mansonia spp.*, *Eretmapodites spp.* and *Culex spp.* mosquitoes, although they are not thought to play a part in YFV transmission due to low virus titres or a short life span. YFV was also isolated from the tick *Amblyomma veriegatum*, which may play a part in the maintenance of the virus through vertical transmission (reviewed in Vainio & Cutts 1998).

31. The non-human primate host range of YFV includes a wide range of monkey species including chimpanzees, marmosets and baboons (reviewed in Vainio & Cutts 1998). YFV has also been detected in mammalian species such as sloths and rodents (Laemmert 1948; de Thoisy et al. 2004) though most of these are not considered hosts as the maximum level of viremia is below transmission thresholds. Neutralising antibodies to YFV have been detected in domestic animals such as camels, cattle, sheep and goats (Findlay et al. 1936; Adu et al. 1990). Experimental animal models to examine YFV replication and disease include mice (Meier et al. 2009), hamsters (Tesh et al. 2001; Xiao et al. 2001) and rhesus monkeys (Monath et al. 1981).

32. *Yellow fever virus* is endemic in South America and Africa (see Figure 2), with the majority of cases and deaths occurring in sub-Saharan Africa, where yellow fever is a major public health problem. South America also experiences periodic yet unpredictable outbreaks of yellow fever. No yellow fever outbreaks have been reported in Asia or Australia; however, the presence of the *A. aegypti* mosquito vector in Northern Australia may facilitate an outbreak should the virus ever be accidentally imported.

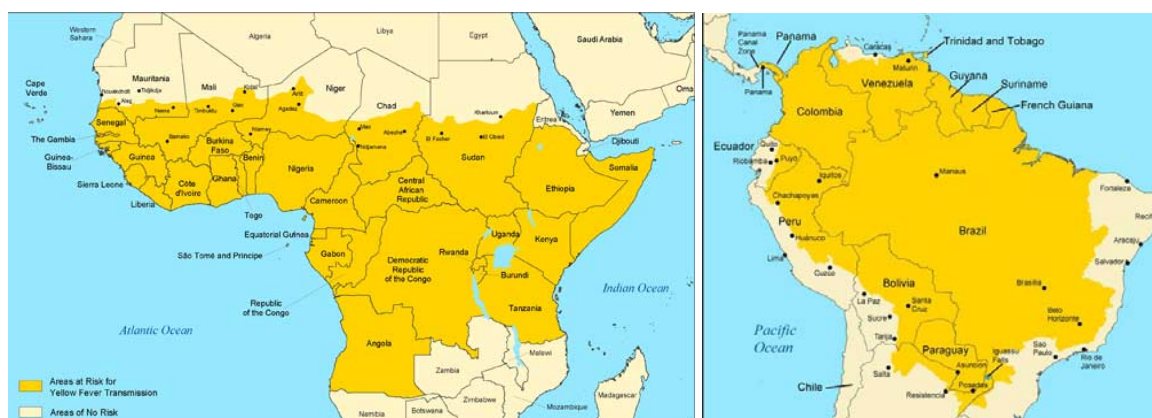


Figure 2 Yellow fever-endemic zones 2009 (Centre for Disease Control and Protection 2010, public domain)

33. *Yellow fever virus* replication takes place through two types of cycles, the jungle or sylvian cycle in which transmission occurs between non-human primates via *Aedes spp.* and *Haemagogus spp.* mosquitoes, and the urban cycle where transmission occurs between humans via the *A. aegypti* mosquito vector. The sylvian cycle is well established in both South America and Africa, and acts a reservoir for the virus, with most human outbreaks occurring as a result of humans intruding on the sylvian cycle. The lack of suitable non-human primate hosts would preclude the possibility of a sylvian cycle being established, should the virus ever enter Australia.

5.2.2 Japanese encephalitis virus

34. *Japanese encephalitis virus* is also an arbovirus and is spread primarily by the *Culex spp.* of mosquitoes, although it has also been isolated from *Aedes* and *Anopheles spp.* (reviewed in Halstead & Jacobson 2003; Halstead & Jacobson 2008) and has a similar mosquito life cycle to YFV (van den Hurk et al. 2009).

35. Waterfowl such as egrets, herons and ducks are considered the primary amplifying hosts of JEV with pigs also contributing to the amplification of the virus. Despite high seroprevalence rates in many mammal species (e.g., cattle, dogs, goats, and rodents), pigs are the only mammals that are considered important in the JEV transmission cycle (van den Hurk et al. 2008; van den Hurk et al. 2009). Long term persistence of JEV in infected bats and reptiles has been demonstrated and it is thought these species may contribute to the maintenance of virus in the absence of mosquito vectors (reviewed in Rosen 1986; van den Hurk et al. 2009).

36. *Japanese encephalitis virus* is endemic throughout Asia with epidemics reported in China, Korea, Japan, Taiwan, Guam, Vietnam, Cambodia, Thailand, India, Nepal and Sri Lanka. Sporadic cases have also been reported in the Philippines, Malaysia, Singapore, Indonesia, Myanmar and Bangladesh (see Figure 3) (Halstead & Jacobson 2003; 2008; van den Hurk et al. 2009; Centre for Disease Control and Protection 2010). *Japanese encephalitis virus* is not considered endemic to Australia, although outbreaks of JEV have been reported in some of the Torres Strait islands and on the mainland (van den Hurk et al. 2001; 2006; 2009).



Figure 3 Japanese encephalitis-endemic zones 2009 (Centre for Disease Control and Protection 2010, public domain)

5.3 Yellow fever virus and *Japanese encephalitis virus* genomic organisation

37. The genomes of YFV and JEV consist of a single strand, positive sense RNA molecule 10 862 and 10 976 nucleotides in length respectively, encoding a single open reading frame (ORF) flanked by short non-translated regions (see Figure 4). The single ORF encodes ten genes, including three structural proteins and seven non-structural proteins. The ten genes are translated as a single polypeptide, cleaved by viral and host proteins, and then processed to produce the individual viral proteins (Ruiz-Linares et al. 1989).

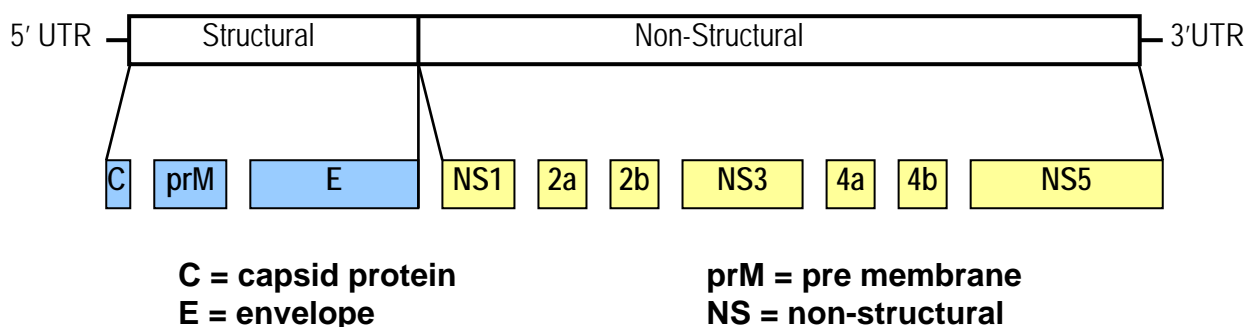


Figure 4 Genomic organisation of *Flavivirus* proteins

38. The genomes of YFV and JEV are identical in gene content and gene order. In order from the translation start site these genes are as follows:

- *C* gene which encodes the capsid protein (C).
- *prM* gene which encodes the pre-membrane protein which is cleaved after viral particle assembly to produce a mature membrane protein (M).
- *E* gene which encodes the envelope protein (E).
- *NS1* gene encodes the non-structural protein NS1 which, in association with NS4a, is required for replicase function, and may have immunomodulatory effects on the host.
- *NS2a* gene encodes the non-structural protein NS2a which is involved in RNA replication and viral assembly, and can act as an interferon (IFN) antagonist in modulating the host immune response.
- *NS2b* gene encodes the non-structural protein NS2b which, in association with NS3, is involved in polyprotein processing.
- *NS3* gene encodes the non-structural protein NS3 which, in association with NS2b, is involved in polyprotein processing and RNA replication.
- *NS4a* gene encodes the non-structural protein NS4a which, in association with NS1, is required for replicase function, is also required for viral assembly, and can act as an IFN antagonist in modulating the host immune response.
- *NS4b* gene encodes the non-structural protein NS4b which is involved in RNA replication, viral assembly, and can act as an IFN antagonist in modulating the host immune response.
- *NS5* gene encodes the non-structural protein NS5 which has been shown to have RNA-dependant RNA polymerase activity and may modulate the host immune response by blocking IFN signalling and inducing expression of interleukin 8 (IL-8).

5.4 Yellow fever virus and Japanese encephalitis virus life cycle

39. The life cycle of a virus involves the transmission of infective viral particles to a host organism, recognition, attachment and entry into the host cells and then replication of viral nucleic acid and protein production, followed by assembly and release of infective virus (see Figure 5). Viruses have co-evolved with their host species and are generally specific for that host organism and infect only certain tissue types within that organism.

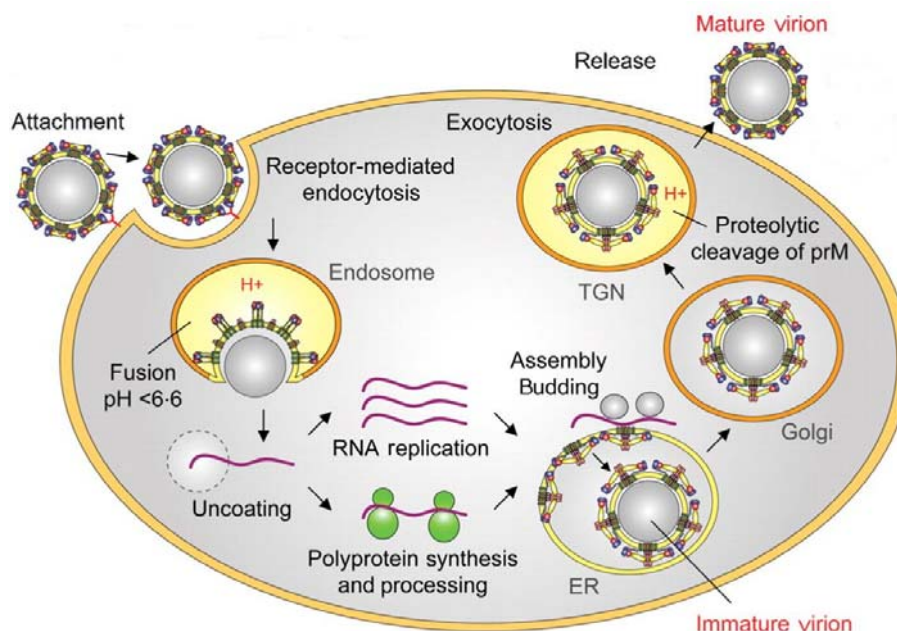


Figure 5 Steps in replication cycle of flaviviruses (taken from Stiasny & Heinz 2006)

40. Infection of a cell by YFV or JEV is initiated by binding to the target cell via interaction of the envelope protein with unidentified receptor molecules on the cell surface. The virus particles are then taken up in clathrin-coated vesicles (Ishak et al. 1988), within which the envelope protein undergoes acid mediated changes in structure leading to fusion with the endosomal membrane and release of the nucleocapsid into the cytoplasm (Stiasny et al. 2009).

41. As the YFV and JEV genomes are single-stranded, positive-sense RNA, they must be transcribed into negative-sense RNA before they can be replicated. Therefore, the viral RNA genome is used both as a template for negative strand synthesis and in the production of viral proteins through the host cellular protein synthesis pathways. Assembly of viral particles occurs in close association with the rough endoplasmic reticulum (ER) (Ruiz-Linares et al. 1989), and viral particles are transported to the cell surface via intracellular vesicles where they are released from the cell through exocytosis (reviewed in Mukhopadhyay et al. 2005; Stiasny & Heinz 2006).

42. In addition to their primary role in viral replication, flavivirus NS2a, NS4a, NS4b and NS5 proteins are thought to inhibit interferon signalling and hence down regulate the host antiviral immune response (Munoz-Jordan et al. 2005).

5.5 Pathology of viral infection

5.5.1 Yellow fever virus

43. The clinical presentation of YFV infection can vary considerably, including asymptomatic infection, abortive infection with non-specific malaise, and potentially lethal pansystemic disease with fever, jaundice, renal failure and haemorrhage. YF has many symptoms in common with a variety of flaviviruses and other fever causing organisms and this can lead to difficulty in diagnosis. Also, as asymptomatic and abortive infections are

rarely reported, the actual level of infection and mortality rates cannot be accurately determined (Monath et al. 2008).

44. The incubation period for YFV ranges from three to six days followed abruptly by the onset of symptoms including fever, chills, headache, myalgia (muscle pain), lumbosacral⁸ pain, anorexia, nausea, vomiting, restlessness, irritability and dizziness. This phase lasts for around three days and corresponds to the time during which virus is present in the blood, and may be followed by a short period of remission in which symptoms may abate for up to 48 hours. In cases of abortive infection, patient recovery may occur at this stage.

45. Approximately 15% of persons infected with yellow fever virus develop moderate or severe disease with return of fever, relative bradycardia⁹, nausea, vomiting, epigastric pain¹⁰, jaundice, reduced urine output and a hemorrhagic diathesis on the 3rd to 6th day after onset. This corresponds to viral clearance from the blood and the production of specific antibodies against YFV antigens.

46. The subsequent disease course reflects dysfunction of multiple organ systems, including the liver, kidneys, and cardiovascular system. The overall picture has the clinical characteristics of the systemic inflammatory response syndrome and multiple organ failure. Death occurs in 20-50% of severe YF cases, and occurs between seven to ten days after the onset of disease. Severe jaundice, hypothermia, delirium, intractable hiccups, hypoglycaemia, stupor and coma are signs of terminal disease (reviewed in Burke & Monath 2001; Monath et al. 2008).

5.5.2 Japanese encephalitis virus

47. The clinical presentation of JEV infection may vary considerably with an estimated 1 in 250 cases leading to symptomatic disease. The primary clinical manifestation is encephalitis, while milder forms include aseptic meningitis, flaccid paralysis and febrile illness.

48. The incubation period for JEV varies from 5 to 16 days. Illness usually begins with an abrupt onset of high fever, chills, gastrointestinal symptoms and headache, followed gradually by dizziness, disturbances in speech or gait or other signs of motor dysfunction. Irritability, vomiting and diarrhoea or acute convulsion may be the earliest signs of illness in an infant or child. Seizures occur in more than 75% of paediatric patients and less frequently in adults. These symptoms are then followed by altered states of consciousness, photophobia and a variety of neurological signs including partial paralysis, tremors or convulsions. Death occurs in 5-40% of severe cases five to ten days after the onset of symptoms, or may occur later from cardiopulmonary complications. Respiratory dysfunction, prolonged fever, prolonged or frequent seizures may be signs of terminal disease.

49. Neuropsychiatric sequelae occur in 45-70% of survivors of severe disease and are more frequent in children. Sequelae include parkinsonism¹¹, convulsive disorders, paralysis, mental retardation and psychiatric disorders (reviewed in Monath & Heinz 1996; Burke & Monath 2001; Halstead & Jacobson 2003; 2008).

⁸ lumbosacral - of or relating to or near the small of the back and the back part of the pelvis between the hips

⁹ bradycardia - abnormally slow heart rate, usually fewer than 60 beats per minute in an adult human

¹⁰ epigastric - pertaining to the epigastrium, the area above the stomach

¹¹ a neurological syndrome characterized by tremor, decreased bodily movement, rigidity, and postural instability

5.6 *Flavivirus environmental stability*

50. Enveloped RNA viruses, such as flaviviruses, are relatively sensitive to heat, desiccation, UV light, household disinfectants and detergents. These viruses cannot not survive for extended periods outside the host or vector organism. Flaviviruses are susceptible to heat and are completely inactivated after 30 minutes at 60°C (Canadian Office of Laboratory Security 2001a; 2001b). The half-life of JEV varies between 28 and 62 minutes at 24°C and 55% relative humidity (Larson et al. 1980). YFV is more fragile, with only 0.08%-0.16% remaining viable after 60 minutes when aerosolised at 27°C with a relative humidity of 30-80% (Mayhew et al. 1968).

5.7 **Yellow fever vaccine strain 17D**

51. YF 17D is a highly attenuated strain of YFV derived from the virulent strain Asibi, which was isolated from a 28 year old West African man named Asibi, in Ghana in 1927. YF 17D was developed at the Rockefeller Institute by serial passages in mouse embryo tissue and minced whole chick embryos (Theiler & Smith 1937; Smith & Theiler 1937). It was first tested in humans in 1936, and large scale trials were carried out in Brazil from 1937. By 1939 over one million Brazilians had been inoculated and vaccination soon became widespread in Africa and South America leading to the eradication of the urban yellow fever cycle. However the sylvian cycle has been maintained and serves as a reservoir for the virus. Recent outbreaks of yellow fever have been linked to a reduction in vaccination in these areas. At least 126 countries currently have YF vaccination requirements for travellers entering, leaving or transiting through areas endemic for YFV (reviewed in Monath et al. 2008).

52. After inoculation YF 17D induces a brief, self-limiting infection characterised by a low level viremia, interferon response and rapid induction of neutralising antibodies. YF 17D is typically asymptomatic or induces only mild symptoms such as headache, myalgia and transient asthenia¹² (Lindsey et al. 2008).

53. It is estimated that over 400 million people have received the YF 17D vaccine with very few reports of severe adverse events such as hypersensitivity to egg proteins or glycine remaining from vaccine production (Kelso et al. 1999), YEL-AVD (Yellow fever vaccine-associated viscerotropic disease) or YEL-AND (Yellow fever vaccine-associated neurotropic disease) (reviewed in Marfin et al. 2005; Lindsey et al. 2008). However, a recent spike in the number of severe adverse events being reported has raised some concerns over the safety and use of the vaccine. Sequencing of vaccines used during this period has not identified any evidence of reversion to the wild type pathogenic strain in these vaccines (Galler et al. 2001; Barban et al. 2007) and the occurrence of these adverse events is thought to be due to pre-existing genetic susceptibility. Risk factors for YEL-AVD include advanced age (over 65 years) and an altered immune status. More recently, patients with history of thymus disease also have been described to be at risk for developing YEL-AVD (Gerasimon & Lowry 2005; Vellozzi et al. 2006; Muñoz et al. 2008; Silva et al. 2009). YEL-AND most commonly occurs in infants and this has led to the recommendation that YF 17D should not be administered to children under the age of 9 months (Cetron et al. 2002).

5.8 **Molecular basis of YF 17D attenuation**

54. The precise basis for the attenuation of YF 17D is not known. An initial study identified 68 nucleotide differences, between YFV Asibi and YD 17D, leading to 32 amino acid substitutions (Hahn et al. 1987), 22 of which occurred in the non-structural proteins present in the GMO backbone. Further studies of YF 17D derivative strains identified 20 conserved amino acid differences (discussed in Monath et al. 2008), 11 of which are present in the

¹² a condition marked by loss of strength in the body

GMO. Four nucleotide changes in the 3' untranslated region (UTR) will also be present in the GMO (Table 2). It has yet to be determined how these amino acid substitutions and nucleotide changes contribute to the attenuated phenotype of the YF 17D strain.

Table 2 Differences between YFV Asibi and YF 17D also present in the GMO

Gene	Genomic Nucleotide	amino acid	YFV Asibi pathogenic strain	YF 17D-204 and YF 17DD vaccine strains
NS1	3371	307	Isoleucine	Valine
NS2a	3860	118	Methionine	Valine
	4007	167	Threonine	Alanine
	4022	172	Threonine	Alanine
	4056	183	Serine	Phenylalanine
NS2b	4505	109	Isoleucine	Leucine
NS3	6023	485	Aspartic Acid	Asparagine
NS4a	6876	146	Valine	Alanine
NS4b	7171	95	Isoleucine	Methionine
NS5	10142	836	Glutamic acid	Lysine
	10338	900	Proline	Leucine
3'UTR	10367		U	C
	10418		U	C
	10800		G	A
	10847		A	C

5.9 Japanese encephalitis vaccine strain SA-14-14-2

55. SA14-14-2 is a highly attenuated strain of JEV derived from the virulent strain SA14, which was isolated from a *C. pipiens* mosquito in Xian China in 1954 (Ni et al. 1994). SA14-14-2 was developed at the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China by serial passages in mouse brain cells, primary hamster kidney cells, chicken embryo cells and suckling mice (Ni et al. 1994). It was first tested in humans in 1979, and was licensed for use in China in 1988 (Halstead & Jacobson 2008). Currently around 110 million doses are produced each year for use within China in their childhood vaccination program (Tsai 2000; Erlanger et al. 2009). Some concerns have been raised concerning side effects resulting from the cell line used to culture the vaccine (World Health Organization 2002). However large scale safety studies suggest the vaccine itself is well tolerated (for example: Bista et al. 2001; Ohrr et al. 2005; Tauber et al. 2007; Sohn et al. 2008).

56. After inoculation SA14-14-2 induces a brief, self-limiting infection and is typically asymptomatic or induces only mild symptoms such as low fevers, myalgia and transient asthenia (Halstead & Jacobson 2008).

5.10 Molecular basis of JE SA-14-14-2 attenuation

57. A comparison of the genomic sequences of SA14-14-2 and its virulent parent, SA14, identified 45 nucleotide differences between the two viruses resulting in 15 amino acid substitutions, six of which occurred in the envelope protein sequence (Nitayaphan et al. 1990) and will be found in the GMO. Further comparison of both genomes with those of the related attenuated strain SA14-2-8 identified 4 conserved changes to the E protein (bold in Table 3) thought to be important to the attenuation of these strains (Ni et al. 1995).

Table 3 Differences between JE SA14 and its attenuated strains which also present in the GMO

Gene	Genomic Nucleotide	amino acid	JE SA14 pathogenic strain	JE SA-14-2-8	JE SA14-14-2 vaccine and GMO
Envelope	1296	107	Leucine	Leucine	Phenylalanine
	1389	138	Glutamine	Lysine	Lysine
	1503	176	Isoleucine	Valine	Valine
	1813	279	Lysine	Lysine	Methionine
	1921	315	Alanine	Valine	Valine
	2293	439	Lysine	Arginine	Arginine

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

6.1 Introduction to the GMO

58. The GM candidate vaccine virus is based on YF 17D that has been modified to contain surface protein genes from JE SA-14-14-2. Table 4 (below) lists the genes inserted into the parent organism.

Table 4 The genes used to alter the antigenic properties of YF 17D

Gene	Function of protein	Source	Intended purpose
<i>Envelope (E)</i>	envelope protein is the major component of the outer surface of virus particle, mediates fusion of the viral particle with the host cell membrane	JEV SA-14-14-2	Elicit an immune antibody response against JEV
<i>pre-Membrane (prM)</i>	membrane protein is the minor component of the outer surface of virus particle, mediates processing and folding of E protein	JEV SA-14-14-2	Elicit an immune antibody response against JEV

59. As discussed in Chapter 1, Section 5.7, YF 17D itself has long been used as a live vaccine as it replicates, but is not virulent, in humans. YFV follows the same route of infection as other mosquito vectored flaviviruses that cause hemorrhagic fever and encephalitis in humans, such as Dengue, WNV and JEV. Therefore YFV could be considered a candidate vaccine vector to deliver Dengue, WNV and JEV antigens, in order to induce appropriate and lasting immune responses without the residual virulence that may be associated with live attenuated Dengue, WNV and JEV (Chambers et al. 1999; Guirakhoo et al. 1999; 2004; Arroyo et al. 2004).

60. The flavivirus E and prM glycoproteins have been identified as the primary antigens that elicit a neutralising, protective antibody response. Therefore the GM vaccine has been modified to replace the YFV prM and E glycoproteins with the corresponding JEV prM and E glycoproteins (Chambers et al. 1999; Guirakhoo et al. 1999) (see Table 4, above).

6.2 Method of genetic modification

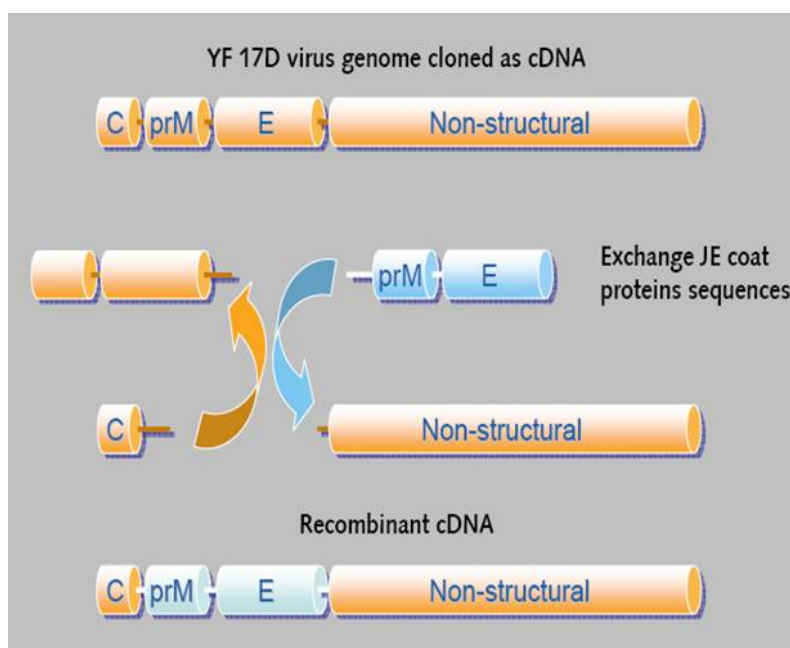


Figure 6 Production of the GM YFV genome

61. The process of genetic modification is represented in Figure 6. In brief, the genomes of both viruses were cloned as cDNA into two plasmids per virus. Sequences encoding the YFV *prM* and *E* genes (YFV nucleotides 482 to 2452) were excised and replaced with sequences encoding the JE SA14-14-2 *prM* and *E* genes (JEV nucleotides 477 to 2477). The plasmids were linearised, ligated and transcribed to produce infectious RNA clones. This RNA was used to transduce tissue culture cells to produce GM virus particles (Chambers et al. 1999). The GM virus particles were serially passaged in tissue culture.

62. The genome of the GM virus was sequenced following tissue culture passage to determine the stability of the inserted DNA. A mutation at nucleotide 935, leading to an arginine to cysteine change at amino acid position 60 in the M protein (M60C), was first identified after passage five, and was dominant in the virus population by passage ten. A second mutation at nucleotide 3161 was also detected; however this mutation is considered silent as it does not correspond to a change in the protein sequence.

63. The M60C mutation enabled better viral growth under tissue culture conditions and was not linked to any change in neurovirulence. Therefore virus containing this mutation was chosen to be developed as the candidate vaccine. The GM virus was purified and amplified to produce the master seed stocks used in the manufacture of the GM vaccine.

64. The effect of the genetic modifications is to create a chimeric virus in which the non-structural proteins involved in viral replication and the capsid proteins are derived from YF 17D, and the surface glycoproteins that mediate binding to host cells and interactions with the host immune system are derived from JE SA14-14-2. The GM vaccine viral particle therefore consists of an YFV nucleocapsid surrounded by an envelope comprised of the JEV M and E glycoproteins (see Figure 7).

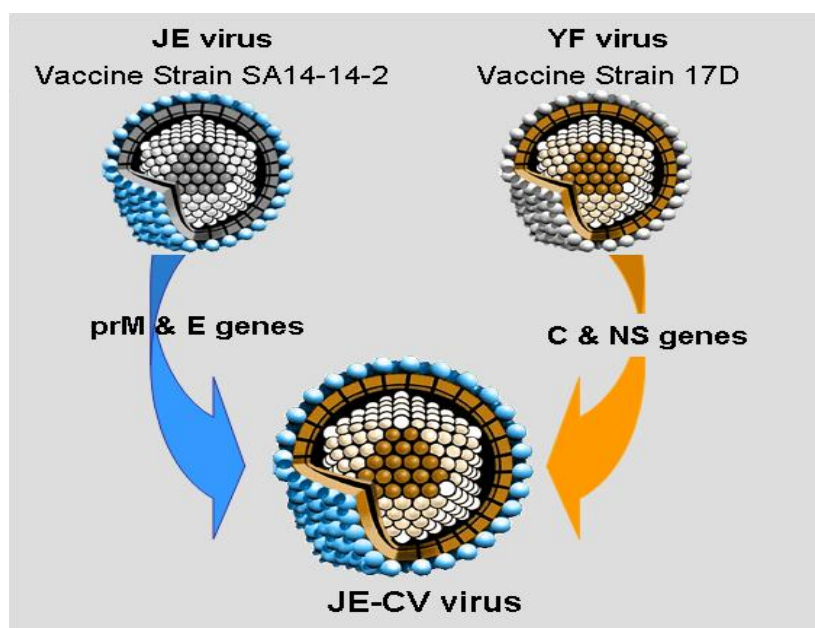


Figure 7 GM viral particle structure

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

65. Full gene sequences have been used for the genetic modifications. The purpose of these modifications is to replace YF 17D proteins with functionally homologous genes from JE SA-14-14-2. The result of these modifications is to produce a replication competent genetically modified virus that is designed to elicit a protective immune response against the JE SA-14-14-2 envelope glycoproteins.

66. The JEV E and prM genes and their encoded proteins perform homologous roles to the YFV genes that they replace, as discussed above. The E and M proteins are the primary antigens eliciting neutralising and protective antibodies as they are displayed on the surface of the virus.

67. The JEV E protein, like the YFV E protein, mediates binding to cellular receptors and fusion of the viral envelope with the cell membrane, and therefore is the primary determinant of host range and cell tropism. When translated in an infected cell it undergoes the same processing and transport as the homologous YFV protein, including activation by cellular proteases.

68. The JEV prM protein, like the YFV prM protein, is known to be important for virus particle assembly and facilitates the proper folding of the E protein to produce mature virions. Prior to viral exit from the cell, the majority of the prM protein is cleaved by a furin like cellular protease to produce the mature M protein which is incorporated into the surface of the viral particles, the remaining pr protein is secreted from the cell and may play a part in modulating the host's immune response to viral infection (Monath et al. 2008).

69. As discussed in Chapter 1, Section 5.3, flavivirus genomes are transcribed as a single polyprotein with transcription initiation and termination sequences encoded in the 5' and 3' untranslated regions respectively. These sequences are external to the introduced genetic sequences and therefore no JEV regulatory sequences were introduced into the GM virus.

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

70. In the context of the GM vaccine the introduced genes have been demonstrated to elicit an immune antibody response without the pathology associated with the viruses from which they are derived. Allergic reactions to components used in the manufacture of YF 17D have been observed (Kelso et al. 1999). However there is no known history of allergic reaction to the YF 17D proteins or genetic material. Similarly there is no known history of allergic reaction to JE SA14-14-2 genes or proteins.

71. No toxicity has been observed in human and animal trials of the GM vaccine (Monath et al. 2002b; 2003; Dean et al. 2005).

6.4 Characterisation of the GMO

6.4.1 Stability and molecular characterisation

72. The parent organism YF 17D, which is the source of the genes encoding the GMO's replication proteins, has a long history of safe use as a vaccine and studies have shown that the attenuating mutations in YF 17D are highly stable with no recorded reversions to a wild type phenotype (Hahn et al. 1987; Galler et al. 2001; Barban et al. 2007; Domingo & Niedrig 2009).

73. As discussed in Chapter 1, Sections 5.8 and 5.10, twenty one attenuating mutations have been identified in the parent organisms and are present in the sequence of the GMO. The genomic RNA of the GM virus has been sequenced. After ten passages in tissue culture cells the recovered virus stably maintained the inserted genes, attenuating mutations and protein expression. However, as noted in Chapter 1, Section 6.2 a M60C mutation was identified after passage five and has been stably maintained in the viral population.

6.4.2 Characterisation of the phenotype of the GM vaccine

74. The phenotype of the GM vaccine has been characterised in model animals such as rats, hamsters, and monkeys (Monath et al. 1999; Dean et al. 2005; Lobigs et al. 2009) and in human clinical trials (Monath et al. 2002b; 2003). Experiments involving direct intracranial inoculation with the GM virus showed no evidence of neurovirulence in monkeys, and significantly reduced neurovirulence in mice compared with the parent YF 17D vaccine. In addition there was no histopathological evidence of damage to the liver or any other organ following inoculation with the virus (Monath et al. 1999; 2005; Dean et al. 2005). These studies also demonstrate the absence of toxicity in mammalian hosts.

6.5 Results of clinical trials with the GM virus

75. The GM vaccine has been tested in clinical trials and demonstrated an acceptable safety profile with no medically significant vaccine-related adverse events when administered to 4000 healthy, adults 18-60 years of age (Monath et al. 2002b; 2003) or seronegative toddlers aged 12 to 24 months.

6.5.1 GMO viremia and shedding in non-human primates following inoculation

76. The vaccine was first tested in rhesus monkeys (*Macacca mulatta*). A total of nine monkeys were inoculated via intracranial (IC) or subcutaneous (SC) injection with varying amounts of the GMO and tested for timing, level and duration of viremia (see Table 5). In all cases viremia peaked soon after inoculation and virus was cleared from the blood by day six (Monath et al. 1999). Urine, faeces and saliva were tested for the presence of the GMO during and directly after the period of peak viremia. No viral RNA was detected, indicating that the virus is not shed in detectable levels.

Table 5 Post inoculation viremia in Monkeys

GMO dose and route of injection	Viremia titre (log ₁₀ pfu/ml) by day post inoculation*					
	1	2	3	4	5	6
4.3log ₁₀ pfu subcutaneous	<1.0	<1.0	1.1	1.7	1.0	<1.0
	<1.0	<1.0	1.0	1.0	<1.0	<1.0
	1.0	<1.0	1.0	1.0	<1.0	<1.0
5.3log ₁₀ pfu subcutaneous	1.0	1.0	1.6	1.0	<1.0	<1.0
	1.3	1.8	1.6	1.1	<1.0	<1.0
	2.0	1.6	1.0	1.0	<1.0	<1.0
6.6log ₁₀ pfu intracerebral	2.7	4.2	2.5	<1.0	<1.0	<1.0
	1.9	3.2	2.2	<1.0	<1.0	<1.0
	3.4	2.9	<1.0	<1.0	<1.0	<1.0

*where 1.0log₁₀ pfu/ml is the limit of detection for the plaque assay

77. A second study was performed in which 12 monkeys were inoculated through:

- (a) subcutaneous injection;
- (b) intradermal injection;
- (c) cutaneous exposure following surface abrasion; or
- (d) cutaneous exposure prior to surface abrasion.

Similar levels of peak viremia were observed (1.7-2.3log₁₀ pfu/ml). However, the onset of viremia was delayed and the duration of viremia was increased following cutaneous delivery (Dean et al. 2005).

6.5.2 *GMO viremia in humans following inoculation*

78. An initial randomised, double-blind, single centre clinical trial involving a cohort of 36 adults aged between 18 and 59 indicated that following inoculation with either 4.0 log₁₀ or 5.0 log₁₀ plaque forming units (pfu), virus was completely cleared from the bloodstream eight days after inoculation and virus levels peaked between days four and five, at less than 200 pfu/ml (Monath et al. 2002b).

79. Similarly, a second randomised, double-blind study involving 99 trial subjects receiving 10-fold graded doses of the GM vaccine (between 1.8 and 5.8 log₁₀ pfu), the parent YF 17D or a placebo indicated that the virus was cleared from the blood within six days and peaked at less than 220 pfu/ml (Monath et al. 2003).

80. A clinical trial of the GM vaccine in healthy, seronegative toddlers aged 12 to 24 months is currently underway. The applicant has indicated that the level of viremia following inoculation is similar to that seen in adults.

81. Other clinical trials, including large scale trials in Australia, USA and Thailand have also been undertaken. The results of these trials have yet to be published; however, Sanofi has indicated that where tested, similar levels of viremia were observed.

6.5.3 *GMO replication in mosquitoes following ingestion or inoculation*

82. An initial study examined the ability of the GM virus to replicate within the known JEV and YFV vectors *A. Aegypti*, *A. Albopictus* and *Culex tirraeniorhynchus*. No GM virus was detected in any of the mosquitoes species following 15 to 30 minutes of feeding on blood meals containing 6.9 log₁₀ pfu/ml of the GM vaccine. Similarly, no viral replication was seen

following intra-thoracic injection of $5.5 \log_{10}$ pfu of GM virus per mosquito (Bhatt et al. 2000).

83. A second study examined the ability of the GM virus to replicate with the Australian mosquitoes *C. Annulirostris*, *C. Gelidus* and *A. vigilax*. These mosquitoes have previously found to be capable of infection with JEV. No GM virus was detected in any of the mosquito species following 2 hours of feeding on blood meals containing $6.1 \log_{10}$ pfu/ml of the GM vaccine. Similarly, no viral replication was seen following intra-thoracic injection of $2.3 \log_{10}$ pfu of GM virus per mosquito (Reid et al. 2006).

84. In both of these studies the potential to replicate in mosquitoes most closely resembled that of YF 17D which also did not replicate in these species, unlike JE SA14-14-2 which was shown to replicate well in the known JEV vectors *A. Aegypti*, *A. Albopictus* and *C. tirraeniorhynchus* (Bhatt et al. 2000; Reid et al. 2006).

6.5.4 GMO replication in non-primate hosts

85. As discussed in Chapter 1, Section 5.2, both parent viruses are capable of replication in a number of avian or mammalian hosts. There are no non-human primates native to Australia. However, waterfowl such as egrets, herons and ducks are common in Australia, as are pigs and bats.

86. The ability of IMOJEV™ to replicate in waterfowl has not been determined. However, experiments with a related YF 17D chimeric virus encoding WNV E and prM proteins showed that it was not able to replicate in chickens (*Gallus gallus*) and fish crows (*Corvus ossifragus*), both of which are known to be amplifying hosts of WNV (Langevin et al. 2003). It was suggested that the inability to replicate in the avian hosts was due to the presence of the YF 17D non-structural proteins in the chimeric virus. These YF 17D proteins are present in the GM virus, and may impact on the ability of the GM virus to replicate in known avian hosts of JEV.

87. An unpublished study by Sanofi Pasteur was unable to detect viral replication in pigs following inoculation with $5.1 \log_{10}$ pfu of IMOJEV™. Additionally, it has been suggested that prior infection or exposure to JEV group viruses endemic to Australia such as MVE, *Alfuy virus*, and *Kunjin virus* is able to protect pigs from infection with JEV (van den Hurk et al. 2008).

Section 7 The receiving environment

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

89. The proposed release involves inoculating infants, young children and adults at medical and clinical facilities throughout Australia. The handling and inoculation of the GM vaccine would be in accordance with the *Australian Immunization Handbook* (National Health and Medical Research Council 2008), the *National vaccine storage guidelines* (Department of Health and Ageing 2005) and the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009) and other relevant guidelines, codes of practice and legislation (see Chapter 1, Section 4.1 for more detail). This is expected to ensure safe receipt, storage, handling, dispensing and disposal of the GM vaccine.

7.1 Relevant environmental factors

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

91. As discussed in Chapter 1, Section 5.2, both parent viruses are transmitted via a mosquito vectors in the *Aedes*, *Anopheles* and *Culex* species. A number of these mosquitoes are present in Australia, particularly in Northern Queensland (Russell 1996; van den Hurk et al. 1998; 2001; Russell et al. 2005; van den Hurk et al. 2008).

92. Also as discussed in Chapter 1, Section 5.2, both parent viruses are capable of replication in a number of avian or mammalian hosts. There are no non-human primates native to Australia. However, waterfowl such as egrets, herons and ducks are common in Australia as are pigs.

93. Flaviviruses are not known to be shed from avian or mammalian hosts or from insect vectors and are rapidly inactivated in the environment.

94. It is expected that the GM vaccine will be administered in clinical or medical facilities under controlled conditions. For locations outside these facilities, physical environmental factors may impact on the possibility and routes of unintended exposure. However, direct blood contact with infectious viral particles, or a bite from a virus infected mosquito vector, is required for exposure to lead to viral replication.

7.2 Presence of related viruses in the receiving environment

95. *Yellow fever virus* and *Japanese encephalitis virus* are not endemic to Australia, although outbreaks of JEV have been reported in some of the Torres Strait islands and Cape York (van den Hurk et al. 2008; 2009). YF 17D has been registered by the TGA for use in Australia as a live viral vaccine and made available to people travelling to areas where YFV is endemic. Similarly JE SA14-14-2 has been registered by the TGA as an inactivated virus vaccine and is available for people travelling to areas where JEV is endemic. Other flaviviruses present in Australia include *Murray Valley encephalitis virus*, *Alfuy virus*, *Kokobera virus* (van den Hurk et al. 2001), *Kunjin virus* and *Hepatitis C virus*, as well as seasonal outbreaks of *Dengue virus* (Liu et al. 2005; 2006; 2008; Fitzsimmons et al. 2009).

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

96. The introduced genes are isolated from organisms that are not present in the Australian environment. However closely related viruses are present in Australia, and therefore some Australians would have been exposed to similar genes. Additionally, both YF 17D and inactivated SA-14-14-2 are registered for use as vaccines in Australia and therefore individuals previously vaccinated against YFV and/or JEV would have been intentionally exposed to some or all of the proteins encoded by the GM vaccine.

Section 8 Australian and international approvals

8.1 Australian approvals of GM *Yellow fever virus* vaccines

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

97. The GM vaccine proposed for commercial release was approved for clinical trials and experimental research in Australia as dealings not involving an intentional release into the environment (DNIR) under licences DNIR 071, DNIR 274, DNIR 319, DNIR 320 and DNIR 366 by the Regulator. The vaccine demonstrated an acceptable safety profile in these clinical trials.

98. The Regulator has also approved clinical trials of a related Yellow fever chimeric virus vaccine against Dengue under DNIR 386.

8.1.2 Approvals by other Australian government agencies

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

100. TGA is the agency with responsibility for approving the use of therapeutic products. Sanofi has applied to the TGA to have the IMOJEV™ vaccine included on the Australian Register of Therapeutic Goods (ARTG). TGA has advised that the vaccine has been registered for use in Australia, as a prescription medicine, by people over the age of 12 months.

8.1.3 Other Australian approvals

101. AQIS has previously approved the importation of IMOJEV™ into Australia as a human therapeutic under AQIS import permits 2005302620, 200515065 and 200515372. Sanofi has indicated that they intend to apply for an AQIS permit to import IMOJEV™ should the TGA approve the inclusion of IMOJEV™ on ARTG.

8.2 International approvals of GM Yellow fever virus vaccines

102. IMOJEV™ was evaluated in nine clinical studies in healthy adult populations in the USA and in Australia, and is also being evaluated in children and toddlers in India, Thailand and the Philippines (Table 6).

Table 6 Overseas applications and approval of trials of the GM vaccine.

Country	Approving Agency
USA	Food and Drug Administration
India	Drugs Controller General of India
Philippines	Department of Health
Thailand	Food and Drug Administration

103. Sanofi has indicated that they have applied to the Thai Food and Drug Administration to have IMOJEV™ registered for use in Thailand.

Chapter 2 Risk assessment

Section 1 Introduction

104. 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 a result of gene technology (Figure 8). Risks are identified within the context established for the risk assessment (see Chapter 1), and takes into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

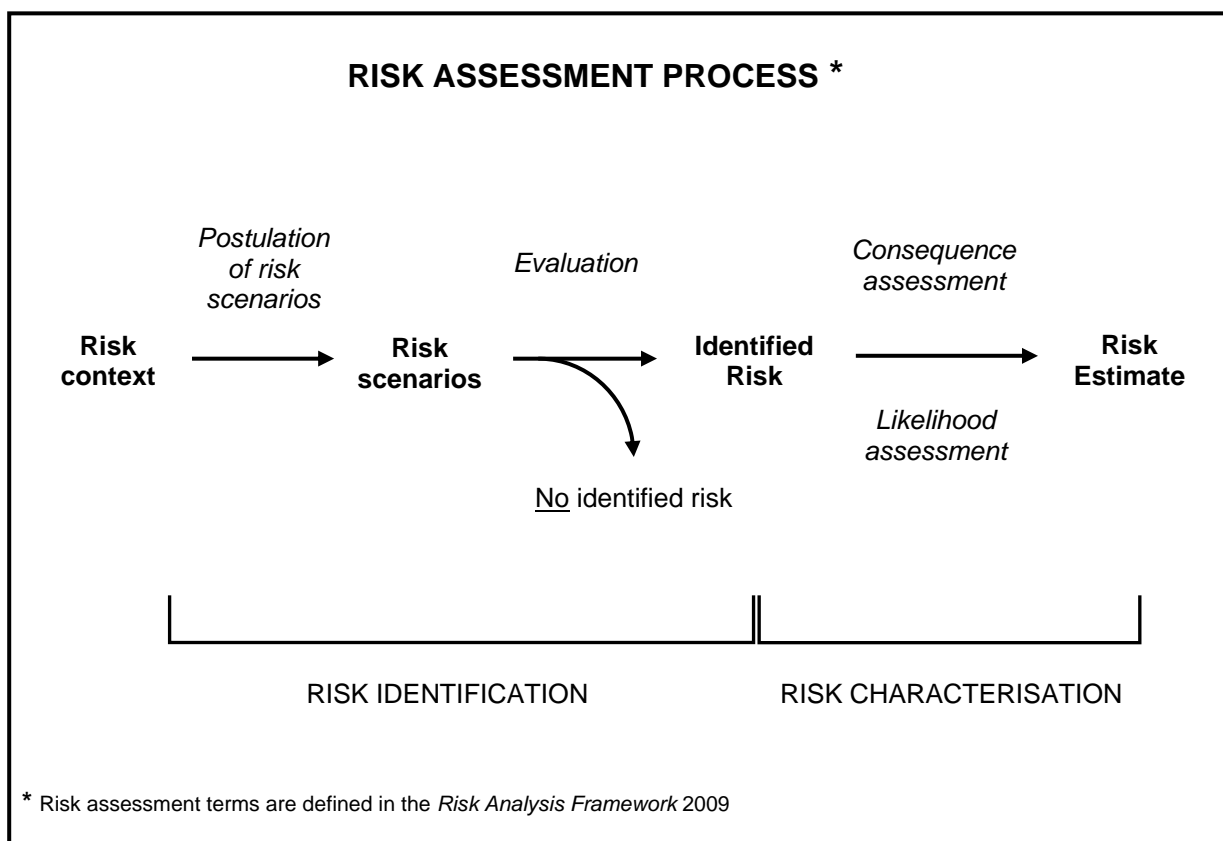


Figure 8 The risk assessment process.

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

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

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

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

Section 2 Risk identification

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

- the proposed dealings, and duration of the dealings, which include importation, possession, supply, transport or disposal of the GMOs
- characteristics of the parent organism
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- clinical management practices for the GMOs.

110. Under section 10 of the Regulations, the Regulator must consider potential risks both in the short term and the long term. Attempts to assign durations for short and long term are not practical and, instead, the Regulator considers the likelihood and consequence of an adverse outcome over the foreseeable future. Long term consideration also involves the identification of specific indicators of risk (see Chapter 3, Section 4) upon which research and testing of credible hypothesis can be undertaken post-licence if a licence were to be issued.

111. As discussed in Chapter 1, Section 3, the TGA has primary regulatory responsibility for assessing patient safety and therefore the risks to people receiving the vaccine will not be considered as part of the evaluation. However, risks resulting from the unintentional exposure of clinical staff while administering the vaccine or resulting from a spill during transport will be assessed.

112. Five risk scenarios were identified and evaluated in detail later in this section. They are summarised in Table 7 where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment.

Table 7 Summary of risk scenarios from dealings with the GM virus.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Accidental exposure of people or animals to the GM virus material containing proteins encoded by the introduced genes	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> • The encoded proteins and their end products have a long history of safe use as vaccines and are unlikely to be toxic or allergenic to people or toxic to other organisms • The proposed classification of the GMO as a Schedule 4 medicine (Prescription Only Medicine) will further reduce exposure of people and other organisms to products of the introduced genes

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.2 Increased disease burden	2. Unintended exposure of people or animals to the GM virus resulting in infection	Increased virulence/severity of symptoms	No	<ul style="list-style-type: none"> The GM virus is not known to cause disease as it is highly attenuated in humans and other potential hosts Transmission of the GM virus is highly unlikely as it does not replicate effectively in any known host or vector The proposed classification of the GMO as a Schedule 4 medicine (Prescription Only Medicine) will further reduce exposure of people and other organisms to the GM virus
Section 2.3 Unintended changes in viral characteristics	3. Changes to the structure and function of the GM virus.	Increased virulence/severity of symptoms	No	<ul style="list-style-type: none"> The introduced genes are closely related to endogenous viral genes and have similar structure and function Standard pharmaceutical monitoring requirements would identify any severe adverse events resulting from unintentional changes in the GMO
Section 2.4 Horizontal transfer of genes or genetic elements to other organisms	4. Presence of the introduced genes in other organisms as a result of gene transfer	Increased virulence/severity of symptoms	No	<ul style="list-style-type: none"> Recombination between flaviviruses is extremely rare and results in viral attenuation The introduced genes are already present in the environment and are available for transfer via natural mechanisms Risk scenarios 1-3 associated with expression of the introduced genes did not constitute identified risks for people or the environment
Section 2.5 Unauthorised activities	5. Use of the GMO outside the proposed licence conditions	Potential adverse outcomes identified in Sections 2.1 to 2.4	No	<ul style="list-style-type: none"> The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator

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

113. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Del Rio 1902).

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

115. A range of organisms may be exposed directly or indirectly to the introduced genes from YFV and JEV. Vaccinated individuals would be intentionally exposed to the GM virus. Clinical staff administering the vaccine or other staff handling the vaccine during transport may be unintentionally exposed through a spill. Waste management workers, insects, birds and animals may be unintentionally exposed through inadvertent disposal of unused vaccine to landfill. Mosquitoes and other biting insects may consume the blood of vaccinated individuals during the time in which GM virus is present in the blood.

Risk scenario 1. Accidental exposure of people or animals to GM vaccine material containing proteins encoded by the introduced genes

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

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

118. Although the viruses are not endemic to Australia, people travelling to areas where the parent viruses are endemic may be exposed to the proteins encoded by the introduced genes. People who have been vaccinated against either JEV or YFV would have been exposed to some or all of the proteins encoded by the GM virus. YF 17D has been administered successfully to over 400 million people worldwide as a vaccine without evidence of toxicity in patients. As discussed in Chapter 1, Section 6.3.1 allergic reactions to chicken proteins and other substances used in the manufacture of YF 17D vaccine have been reported (Marfin et al. 2005; Lindsey et al. 2008). However, no allergic response to the proteins encoded by YF 17D has been observed. Similarly JE SA 14-14-2 has been licensed for use in China since 1988 and is administered to approximately 50 million children each year with no evidence of allergic or toxic reactions (Marfin et al. 2005; Halstead & Jacobson 2008). No information was identified to suggest that the proteins encoded by the introduced genes are toxic or allergenic to people or other organisms.

119. Japanese Encephalitis vaccines are currently classified as a Schedule 4 medicine (Prescription Only Medicine) under the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009). If the GM vaccine is also included in Schedule 4, as proposed by the applicant, access to the GM vaccine would therefore be limited to pharmacy staff, medical practitioners and health care professionals and the GM vaccine would be stored in restricted access areas. The GM vaccine would be transported by commercial couriers in accordance with the *Australian Code of Good Wholesaling Practice For Therapeutic Goods For Human Use* (Therapeutic Goods Administration 1991) and also the WHO *Good distribution practices for pharmaceutical products* (World Health Organisation 2010). Unused vaccine and waste would be disposed of through standard clinical waste disposal methods such as steam sterilisation or incineration (Australian Capital Territory 1991a; New South Wales 1997; Queensland 2000; Victoria 2000; Tasmania 2000a; West Australia 2004; Northern Territory 2009; South Australia 2009). Spills would be disinfected and cleaned up according to standard clinical procedures. Therefore there is very little potential for accidental exposure to the GM virus.

120. Additionally, the GM vaccine is not expected to be shed from vaccinated individuals, and transmission could only occur through direct blood contact shortly after inoculation. As discussed in Chapter 1, Section 6.5, the GM virus has been shown to replicate to low levels in inoculated individuals and not at all in mosquitoes. Therefore accidental exposure through transmission from vaccinated individuals is highly improbable. Exposure of animals to the GM vaccine would also be highly improbable.

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

2.2 Increased disease burden from the GM virus

122. Baseline information on the characteristics of, and the factors limiting transmission of, YFV and JEV are given in Chapter 1. In summary, neither YFV nor JEV are considered endemic to Australia; however, viruses closely related to JEV are present. Both viruses cause severe disease, and in some cases death, in human hosts. YFV can also cause a comparable disease in some non-human primates. Both viruses are generally asymptomatic in other mammalian hosts. The parent vaccine strains YF 17D and SA 14-14-2 are highly attenuated and show reduced replication in human and mammalian hosts. Flaviviruses are transmitted through the bite of an infected invertebrate vector, and human to human transfer has only been demonstrated through the transfusion of infected blood. Flaviviruses are inactivated by exposure to the environment for less than an hour.

123. Pathways that could lead to an increased disease burden from the GM virus include transmission of the GM virus to susceptible people or other organisms, expression of the introduced genes conferring lower infectious dose, increased shedding, or increasing the number of susceptible host organisms, compared to the parent YFV or JEV viruses.

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

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

125. The GM virus has been shown to be highly attenuated in both humans and non-human primates. Accidental exposure to the GMO is expected to result in the same response as intentional inoculation with the vaccine; typically this would be asymptomatic, but may include localised inflammation, mild headache and/or mild fever of short duration. Additionally both parent viruses are also highly attenuated in humans, and not known to cause significant disease. YF 17D has a long history of safe use as a vaccine and studies have shown that the attenuating mutations in YF 17D are highly stable with no recorded reversions to a wild type phenotype (Hahn et al. 1987; Galler et al. 2001; Barban et al. 2007; Domingo & Niedrig 2009).

126. Flaviviruses are not known to be shed from infected hosts; instead transmission occurs through the bite of an infected mosquito vector or through direct blood contact during the viremic period. As discussed in Chapter 1, Section 6.5, no virus shedding was detected in urine, faeces or saliva of infected monkeys during the period of peak viremia. The GM virus has been shown to replicate to very low levels in inoculated individuals and not at all in mosquitoes. Similarly, although transmission of the parent YF 17D vaccine through blood transfusion has been demonstrated (Lederman et al. 2010), standard blood donation screening practices would prevent vaccine recipients from donating blood during their expected period of viremia. Therefore exposure of humans or suitable non-human hosts through transmission from vaccinated individuals is highly improbable.

127. As discussed in Chapter 1, Section 6.5.4, the applicant has indicated that the virus is unable to replicate in pigs, the JEV mammalian host, and experiments with a related WNV/YF 17D chimeric virus did not detect any viral replication in known avian hosts (Langevin et al. 2003). Therefore, even if pigs or suitable avian hosts were accidentally exposed to the GMO, it is not expected that the GMO would be able to replicate or persist within the environment.

128. Japanese Encephalitis vaccines are currently classified as a Schedule 4 medicine (Prescription Only Medicine), under the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009). If the GM vaccine is also included in Schedule 4, as proposed by the applicant, access to the GM vaccine would therefore be limited to pharmacy staff and medical practitioners, and the GM vaccine would be stored in restricted access areas. The GM vaccine would be transported by commercial couriers in accordance with the *Australian Code of Good Wholesaling Practice For Therapeutic Goods For Human Use* (Therapeutic Goods Administration 1991) and also the WHO *Good distribution practices for pharmaceutical products* (World Health Organisation 2010). Unused vaccine and waste would be disposed of through standard clinical waste disposal methods such as steam sterilisation or incineration (Australian Capital Territory 1991a; New South Wales 1997; Queensland 2000; Victoria 2000; Tasmania 2000a; West Australia 2004; Northern Territory 2009; South Australia 2009). Spills would be disinfected and cleaned according to standard clinical procedures. Therefore there is very little potential for accidental exposure of humans, pigs or avian hosts to the GM virus.

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

2.3 Unintended changes in viral characteristics

130. Single strand, positive sense RNA virus genomes code for a variety of genes and the gene products of individual genes may display pleiotropy¹³, performing more than one function in the process of virus infection and replication (Hanley et al. 2003; Santiago & Sanjuán 2007). Gene technology has the potential to cause unintended effects by introducing a gene product that could affect multiple traits. Such pleiotropic effects may include:

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

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

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

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

133. Human, animal and insect trials involving the GM virus and closely related GM viruses expressing Dengue virus or West Nile virus *E* and *prM* genes, have not demonstrated unexpected changes in the characteristics of the GM virus resulting from the introduced genes (Monath et al. 1999; Chambers et al. 1999; Guirakhoo et al. 1999; Bhatt et al. 2000; Guirakhoo et al. 2000; 2001; Johnson et al. 2002; Guirakhoo et al. 2002; Monath et al. 2002a; 2002b; 2003; Johnson et al. 2004; Guirakhoo et al. 2004; Arroyo et al. 2004; Beasley et al.

¹³ Pleiotropy is the genetic effect of one gene on apparently unrelated, multiple phenotypic traits (Kahl 2001).

2004; Monath et al. 2005; Brandler et al. 2005; Dean et al. 2005; Chambers et al. 2006; Higgs et al. 2006; Reid et al. 2006; Guirakhoo et al. 2006; Guy et al. 2008a; 2008b; 2010). Other considerations relevant to viral characteristics in relation to expression of the introduced genes, have already been discussed in Risk Scenarios 1 to 3, and were not considered identified risks.

134. Due to known similarities in sequence and structure, the replacement of YF 17D *E* and *prM* genes with the corresponding JE SA-14-14-2 *E* and *prM* genes is not expected to alter the transcription or translation of YF 17D genes downstream of the insertion point. Similarly the post translational processing of the encoded proteins would not be altered by the introduced gene sequences. Likewise, the JEV proteins are known to be functionally homologous to those from YFV and the substitution of these proteins is unlikely to result in the acquisition of additional unexpected properties.

135. The applicant has applied to the TGA to have the GM virus included on ARTG and Japanese Encephalitis vaccines are currently classified as a Schedule 4 medicine (Prescription Only Medicine) under the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009). As such the applicant will be required to monitor and report any serious adverse reactions occurring as a result of the vaccination with the GMO (Therapeutic Goods Administration 2005). Additionally standard licence conditions require the applicant to inform the Regulator if they become aware of any 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.

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

2.4 Horizontal transfer of genes or genetic elements to other organisms

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

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

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

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

140. Horizontal gene transfer by recombination between flaviviruses is extremely rare. In order for recombination to occur a single cell must be co-infected with at least two separate virus strains. As discussed in Chapter 1, Section 6.5 the GMO produces only low levels of transient viremia following inoculation. As discussed in Chapter 1, Section 7.2 JEV and YFV are not present in Australia, although limited incursions of JEV have been recorded. It is possible that a traveller from an area in which JEV is endemic could enter Australia during a period of asymptomatic viremia, although this typically lasts for less than a week in such

infections, or a new incursion of JEV into Australia could occur. However, as the vaccine is intended for prophylactic rather than therapeutic use, it is unlikely that an individual would be exposed to the GM vaccine while infected with JEV.

141. Outbreaks of the related MVEV and *Kunjin virus* occur very rarely and affect only a low proportion of the population (Liu et al. 2005; 2006; 2008; Fitzsimmons et al. 2009). Seasonal outbreaks of *Dengue virus* can occur as a result of the virus being imported by infected travellers. However, spread within Australia has been limited by response programs put in place by State and Territory governments, and consequently Dengue is not considered endemic in Australia (Beebe et al. 2009; Russell et al. 2009). Therefore co-infection with the GMO and a related flavivirus is unlikely to occur.

142. While a number of phylogenetic sequence analysis studies have suggested that recombination in JEV and Dengue virus may have occurred in the past (Worobey et al. 1999; Holmes et al. 1999; Aaskov et al. 2007; Chen et al. 2008; Baillie et al. 2008; Perez-Ramirez et al. 2009), successful recombination only been reported once in experiments designed to promote and detect recombination events (Chuang & Chen 2009). The study involved co-infection of human and insect cells with two strains of JEV and used restriction fragment length polymorphisms to identify recombinant viruses. The mechanism of recombination is thought to involve switching of the RNA polymerase between the different genomes during transcription. A second study detected two aberrant recombination events resulting in the duplication of around 600 nucleotides and lead to impaired replication in the resulting viruses (Taucher et al. 2010).

143. Artificially produced recombinants between a number of flaviviruses have all shown reduced pathogenesis and/or virulence when compared with the parent viruses (for example: Pletnev et al. 1992; Arroyo et al. 2001; Pletnev et al. 2002; Mathenge et al. 2004; Guy et al. 2008a; Domingo & Niedrig 2009). Artificial recombinants between the GMO and the related *Kunjin virus* were attenuated compared to the wild type *Kunjin virus* (Pugachev et al. 2007). Additionally, experiments with a highly related Dengue virus/YF 17D chimeric virus showed that substitution of YF 17D sequences with those from the virulent YF Asibi strain did not enhance the chimeric virus's virulence, pathogenicity or ability to replicate in mosquitoes (McGee et al. 2008a; 2008b). Therefore it is not expected that recombination between the GMO and a circulating flavivirus strain would lead to virus which is more pathogenic or virulent than the circulating wild type flavivirus.

144. HGT could also result in the presence of the introduced genes in bacteria and in animals or other eukaryotes. However, the introduced sequences were isolated from organisms already widespread in the environment (See Chapter 1, Section 7.2) and already available for transfer via natural mechanisms. Additionally flaviviruses are RNA based viruses which replicate in the cytoplasm of the host cell and do not produce DNA as part of their lifecycle (Burke & Monath 2001; Lindenbach et al. 2007) and therefore incorporation of viral sequences into the DNA genome in the nucleus of the cell would not be expected occur.

145. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes rather than horizontal gene transfer per se (Keese 2008). If the introduced genes or their end products are not associated with harm to people or other organisms then even in the unlikely event of HGT occurring, they should not pose risks to humans, animals or the environment. Conclusions reached for Risk scenarios 1 - 3 associated with the expression of the introduced genes did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

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

2.5 Unauthorised activities

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

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

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

Section 3 Risk estimate process and assessment of significant risk

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

150. None of the risk scenarios were considered to lead to an identified risk that required further assessment.

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

- long history of safe use of the parent vaccine viruses containing the same proteins or sequences encoded by the introduced genes with no evidence of harm to otherwise healthy people
- limited ability of the GM virus to replicate in humans and other animals
- limited ability of the GM virus to replicate in mosquito vectors
- limited ability and opportunity for the GM vaccine to transfer the introduced genes

152. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM vaccine into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment¹⁴.

¹⁴ As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 8 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Section 4 Uncertainty

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

154. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁵. For clinical trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict exposure to the GMO and its genetic material in the environment, rather than necessarily to treat an identified risk.

155. For DIR 098 which involves commercial release of the GMO, uncertainty exists in relation to the potential for occurrence of rare serious adverse reactions resulting from inoculation with the GMO, as these may not become apparent during clinical trials with limited numbers of patients. As a result, it is expected that the conditions of registration on ARTG will include a requirement for the applicant to inform the TGA of any serious adverse reactions resulting from the use of the GM vaccine (Therapeutic Goods Administration 2005).

156. Additional data, including information to address this uncertainty, may be required to assess any future application for dealings with the GM virus to be included on the GMO register.

¹⁵ A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2009) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

Chapter 3 Risk management plan

157. 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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through proposed licence conditions. The risk management plan informs the Regulator's decision-making process. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

158. 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.

159. All licences are required to be 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 contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

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

Section 2 Responsibilities of other Australian regulators

161. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, TGA, National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies¹⁶.

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

163. Sanofi has applied to the TGA to have IMOJEV™ included on the Australian Register of Therapeutic Goods (ARTG). The OGTR liaised with TGA during the assessment of this licence application. TGA has advised that the vaccine has been registered on the ARTG for use in Australia by people over the age of 12 months.

¹⁶ More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework (OGTR 2009)* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>

164. AQIS has previously approved the importation of IMOJEV™ into Australia as a human therapeutic under AQIS import permits 2005302620, 200515065 and 200515372. Sanofi has indicated that they intend to apply for an AQIS permit to import IMOJEV™.

Section 3 Risk management

3.1 Specific risk management considerations

165. Five risk scenarios listed in Chapter 2 were considered in the context of the proposed commercial release and the receiving environment (Chapter 1, Section 7). The risk assessment of the risk scenarios concluded that the risks to people and the environment from the proposed commercial release of GM vaccine are **negligible**. The *Risk Analysis Framework* (OGTR 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

166. The GMO is intended to be released as a registered therapeutic and the proposed commercial release is also contingent on registration of the GMO by the TGA on the ARTG. Japanese Encephalitis vaccines are currently classified as a Schedule 4 medicine (Prescription Only Medicine) under the *Standard for the Uniform Scheduling of Drugs and Poisons* (Poisons Standard 2009). As discussed in Chapter 1, Section 3, state and territory legislation (West Australia 1964; New South Wales 1966; Victoria 1981; Northern Territory 1983; South Australia 1984; Queensland 1996; South Australia 1996; Tasmania 2000b; Victoria 2006; Australian Capital Territory 2008) provide criminal penalties for non-secure storage or improper supply of Schedule 4 medicines. This is considered adequate to ensure that access to the GMO would be restricted to those with the appropriate authorisation. Therefore the Regulator does not need to impose additional conditions to limit access to the GMO. It is also relevant that the transport, storage and administration of the GM vaccine would be in accordance with the *National Immunization Handbook* (National Health and Medical Research Council 2008), *National vaccine storage guidelines: Strive for 5* (Department of Health and Ageing 2005), *Australian Code of Good Wholesaling Practice For Therapeutic Goods For Human Use* (Therapeutic Goods Administration 1991) and also the WHO *Good distribution practices for pharmaceutical products* (World Health Organisation 2010). Although there is no legislation enforcing these guidelines, appropriate handling of the GMO during transport is required to ensure the GM vaccine remains viable.

167. Additionally the appropriate disposal of clinical waste and unused pharmaceuticals is regulated through relevant state and local government OH&S and environmental protection legislation (Australian Capital Territory 1991a; Australian Capital Territory 1991b; South Australia 1993; New South Wales 1997; Queensland 2000; Victoria 2000; Tasmania 2000a; West Australia 2004; Northern Territory 2009; South Australia 2009). Typically clinical waste is destroyed through high temperature incineration or sterilised through an appropriate steam or temperature regime followed by burial in designated land fill sites. These methods are considered appropriate to dispose of the GMO and therefore no further conditions related to disposal are required.

168. The conditions expected to be imposed by the TGA for a registered live attenuated viral vaccine, the guidance provided by the above documents and the relevant waste disposal legislation are considered sufficient to maintain the risk context for the proposed release.

3.2 General risk management considerations

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

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

3.2.1 Applicant suitability

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

- any relevant convictions of the applicant (both individuals and the body corporate);
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country;
- the applicant's history of compliance with previous approved dealings; and
- the capacity of the applicant to meet the conditions of the licence.

171. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Sanofi suitable to hold a licence.

172. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

173. Sanofi must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2.2 Testing methodology

174. Sanofi is required to provide a method to the Regulator for the reliable detection of the presence of the GMO and the introduced genetic materials in a recipient organism. This instrument would be required within 30 days of the issue date of the licence.

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

175. The persons covered by the licence would be any person in Australia, including the licence holder. However, as discussed previously, the GMO has been registered on ARTG and listed as a Schedule 4 medicine and therefore access to the GMO would be restricted and it would only be made available on prescription by a registered medical practitioner.

3.2.4 Reporting structures

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

- any additional information regarding risks to the health and safety of people or the environment associated with the release;

- any changes to the conditions of registration, relating to the pattern of usage of the GMO, approved by the TGA;
- any contraventions of the licence by persons covered by the licence; and
- any unintended effects of the commercial release.

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

3.2.5 Monitoring for Compliance

178. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

179. 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 health and safety of people or the environment could result.

Section 4 Post release review

180. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but 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.

181. For the current application for a DIR licence, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will be achieved through post release review (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).

182. 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

183. Any member of the public can report adverse experiences/effects resulting from an intentional release to the OGTR through the Free-call number (1800 181 030), fax (02 6271 4202), 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 the review of the RARMP (see 4.3 below) as well as the risk assessment of future applications involving similar GMO(s).

184. Adverse reactions resulting from the use of an approved medicine can also be reported to the TGA through the Consumer Adverse Medicine Events Line (1300 134 237), or direct to

the TGA through the Free-call number (1800 044 114), fax (02 6203 1616), mail (Office of Medicines Safety Monitoring, TGA, PO Box 100, Woden ACT 2606) or by email <adr.reports@tga.gov.au>. For more information please refer to the TGA website <<http://www.tga.gov.au/problem/medicines.htm>>.

4.2 Requirement to monitor specific indicators of harm

185. Additional specific information on the proposed 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. Specific indicators of harm may also be identified during later stages, eg following the consideration of comments received on the consultation version of the RARMP, or following the release of the GMO if a licence were issued by findings from either of the other components of PRR.

186. 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.

187. The triggers for this component of PRR may include:

- risk estimates greater than negligible
- uncertainty in the risk assessment.

188. None of the events discussed in Chapter 2 gave rise to an identified risk, and no specific indicators of harm have been identified in this RARMP for application DIR 098.

4.3 Review of the RARMP

189. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would be desktop-based and take into account any relevant new information or may be triggered by findings from either of the other components of PRR. The purpose of the review would be to ensure the findings of the RARMP remained current and the timing of the review would be determined on a case-by-case basis. 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 needed managing, this could lead to review of the risk management plan and changes to the licence conditions.

Section 5 Conclusions of the RARMP

190. The risk assessment concluded that the dealings associated with this commercial release of the GM vaccine as a prescription medicine pose negligible risks to the health and safety of people or the environment as a result of gene technology.

191. The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to allow appropriate oversight of the ongoing release.

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

Consequence

adverse outcome or impact of an activity

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

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

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

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

Harm

adverse outcome or impact

Likelihood

chance

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Risk

chance of harm from an activity

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

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

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

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

Risk analysis

overall process of risk assessment, risk management and risk communication

Risk analysis framework

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

Risk assessment

overall process of hazard identification and risk characterisation

Risk characterisation

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

Risk communication

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

Risk context

parameters within which risk is assessed, managed and communicated

Risk criteria

terms of reference against which the significance of risk is evaluated

Risk estimate

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

Risk evaluation

process of determining if risk requires risk treatment

Risk identification

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

Risk management

mechanisms to control and mitigate risk

Risk management plan

scheme for managing risk posed by dealings with a GMO

Risk scenario

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

Risk treatment

process of selection and implementation of measures to reduce risk

Stakeholders

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

States

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

Uncertainty

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

Appendix B. Summary of issues raised in submissions received from prescribed experts, agencies and authorities¹⁷ on any matters considered relevant to the preparation of a Risk Assessment and Risk Management Plan for DIR 098

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

Summary of comments received	Where considered in RARMP
There have been no reports of adverse effects on the environment from clinical trials of IMOJEV™ in Australia under DNIRs 071, 274, 319, 320 and 366.	Chapter 1, Section 6.5 Risk scenario 1 and Risk scenario 2.
The vaccine should be clearly labelled to for human use only.	It has been proposed that the vaccine will be listed as a Schedule 4 – Prescription Only Medicine. Labelling of pharmaceuticals is regulated by the TGA.
IMOJEV™ is derived from viruses which already circulate in the environment, and is attenuated compared to these viruses.	Chapter 1, Sections 5 to 7.
IMOJEV™ exhibits low multiplication in humans and other hosts.	Chapter 1, Section 6.5 Risk scenario 1 and Risk scenario 2.
The potential for transmission of the GM vaccine to people, animals and insects should be considered	Chapter 1, Section 6.5 Risk scenario 1 and Risk scenario 2.
IMOJEV™ does not spread without a mosquito vector, is not shed from the body of an infected host and does not replicate in mosquitoes.	Chapter 1, Section 6.5 Risk scenario 1 and Risk scenario 2.
IMOJEV™ will be administered under controlled circumstances by health professional located in Australian medical facilities. Unintended direct release (spills) could be managed by standard disinfection procedures.	Chapter 1, Section 4.1.
IMOJEV™ is fragile outside the host cell with limited survival in the environment.	Chapter 1, Section 5.6.
The potential for viral recombination with wild type flavivirus should be considered	Chapter 1, Section 6.4 and Risk scenario 4.
Flaviviruses can undergo random mutation; however, research has shown that IMOJEV™ appears genetically stable.	Chapter 1, Section 5.6 and Risk scenario 3 and Risk scenario 4.
Flavivirus recombination is rare and recombined viruses are attenuated compared to either parent virus.	Chapter 1, Section 6.4 and Risk scenario 4.

¹⁷ GTTAC, State and Territory Governments, Australian Government agencies, Local Councils and the Minister for the Environment Protection, Heritage & the Arts.

Appendix C. Summary of issues raised in submissions received from prescribed experts, agencies and authorities on the consultation RARMP for DIR 098

The Regulator received a number of submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and where they are addressed in the consultation RARMP, are grouped and summarised below.

Summary of comments received	Where considered in RARMP
Indicated that it was unclear from the RARMP whether the vaccine will be used for adults or for children over the age of 12 months	The TGA has primary responsibility for assessing the risks associated with the use of the GMO as a vaccine in humans and is responsible for determining the appropriate age restriction for this vaccine. The RARMP was modified to reflect the advice provided by the TGA and to clarify that use would be permitted to people over the age 12 months. Chapter 1, Section 4
Notes that potential pathways to harm for animals would involve human shedding of the GMO and transmission to animals. Also notes that : <ul style="list-style-type: none"> • GMO is cleared from the blood within 6 days • virus titres in blood are low • no evidence of replication in mosquitoes • GMO does not appear to replicate in avians and pigs Therefore agrees with conclusion of negligible risk to animals in the environment	Noted. Chapter 1, Section 6.5 Risk scenario 1 and Risk scenario 2.

Appendix D. Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 098

The Regulator received three submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised a number of issues which were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

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

Type: I: individual, O: Organisation

Issue raised: A: Applicant suitability; B: Benefits of gene technology; C: Point of clarification; GS: Genetic Stability; H: Human health; HGT: Horizontal Gene Transfer; I: Inadequate information; LC: Licence Condition; LT: Long Term effects; RA: Risk Assessment; U: Unintended effects; VR: Viral Recombination; VT: Viral Transmission.

Sub. No:	Position	Type	Issue	Summary of issues raised	Consideration in RARMP	Comment
1	x	I	RA	Does not support the release of the GMO. Concerned over the terminology used in the RARMP, particularly the use of negligible for risks that could occur.	-	A large number of potential risk scenarios were considered during the risk assessment process. However, only those scenarios involving a plausible pathway to actual harm were discussed in the RARMP. Evaluation of the risk scenarios concluded that it is highly unlikely that harm would occur as therefore risks to people and the environment were considered to be negligible.
2	x	O	B	Does not support the release of the GMO. Questions the need for, and benefits of, a GM vaccine for Japanese encephalitis given the existence of a non-GM vaccine.	-	The frame of reference for risk assessment is defined by the Act, which requires the Regulator to consider risks to human health and safety and the environment posed by or as a result of gene technology and to manage any identified risks. The need for, and benefits of, the GM JEV vaccine are outside the scope of issues to which the Regulator must have regard when deciding whether or not to issue a licence.

Sub. No:	Position	Type	Issue	Summary of issues raised	Consideration in RARMP	Comment
2 (Cont)	x	O	I	Questions the quality, independence and sufficiency of information contained in the application. States that the onus of proof for the safety of GMOs lies with the proponents not with the critics.	-	The Regulator was satisfied that the applicant supplied all the information which was required for the assessment of this application. This information was assessed for its relevance, quality and value as evidence to support the preparation of the RARMP. In addition to information supplied by the applicant, the RARMP also considered relevant peer reviewed published scientific literature. Furthermore, the RARMP and its conclusions were subject to review and comment by a range of experts, agencies and authorities and relevant comments were taken into account prior to finalising the RARMP which formed the basis of the decision to issue a licence.
			RA	Questions whether the five risk scenarios discussed in the RARMP were broad enough to identify and evaluate all reasonably possible risks	Chapter 2, Section 1 and Section 2	The risk identification process for this RARMP considered a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. However, only those scenarios involving a plausible pathway to actual harm were discussed in the RARMP.
			RA	Questions the use and applicability of substantial equivalence in the assessment of risks posed by the release.	-	'Substantial equivalence' is a process used in the assessment of GM foods. This process is not used for GM vaccines. Instead, the potential vaccine is required to pass the same pre-clinical and clinical trial process of testing for safety and efficacy as any non-GM vaccine. The GM vaccine has been tested in clinical trials in Australia, the USA and Thailand and demonstrated an acceptable safety profile. Additionally, experiments were carried out which demonstrated that the GMO was unable to replicate in mosquitoes or animals and had little potential for transmission, and therefore persistence, within the environment. The risk assessment process included comparisons with both pathogenic and attenuated vaccine strains of JEV and YFV as these most accurately represented the potential range of characteristics provided by the genetic material present in the GMO.
			I	Questions whether there is sufficient evidence to prove the safety and efficacy of the GM vaccine.	Chapter 1, Section 3 and Chapter 3, Section 2	The efficacy of the GM vaccine for use in human is outside scope of issues to which the Regulator must have regard when deciding whether or not to issue a licence. TGA has primary regulatory responsibility for assessing the quality, efficacy and patient safety of the GM vaccine for use in humans. The Regulator notes that the TGA has assessed risks to patients and will manage any risks identified.

Sub. No:	Position	Type	Issue	Summary of issues raised	Consideration in RARMP	Comment
2 (Cont)	x	O	GS	Questions whether the GMO reliably displays the same benign characteristics of the non-GM parents and points out that a GM mousepox was shown to have increased pathogenicity when compared to the wild type virus.		The GM JE vaccine has been tested extensively in a range of animal hosts and in clinical trials in people. No increase in virulence or pathogenicity was observed in any of the experiments.
			I	Requests more substantial scientific evidence on: <ul style="list-style-type: none"> the long history of safe use of the parent vaccines the limited ability of the GM virus to replicate in humans and other animals the limited ability of the GM virus to replicated in mosquitoes the limited ability and opportunity for the GM virus to transfer the introduced genes 	Chapter 1, Sections 5.7-5.10 and 6.5 Risk scenarios 1, 2 and 4	After critically evaluating all of the relevant scientific information currently available, the Regulator was satisfied that the data supports the conclusions presented in the RARMP. Risks to people and to the environment were considered to be negligible on the basis of the scientific information presented and referenced in the RARMP.
				Notes that the family <i>Flaviviridae</i> contains a number of significant human pathogens.	Chapter 1, Section 5.1	Noted.
			LT, H	Considers that more information is required on the long term safety impacts of the vaccine.	Chapter 3, Section 3.2 and Section 4	The licence conditions include a requirement for Sanofi to report any 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 and also any unintended effects of the dealings authorised by the licence. The GTR has the ability to take further regulatory actions if required to protect people and the environment. Additionally the TGA has a pharmacovigilance program which requires medicine sponsors to inform the TGA of any serious adverse reactions resulting from the use of registered medicines including prescription medicines.

Sub. No:	Position	Type	Issue	Summary of issues raised	Consideration in RARMP	Comment
2 (Cont)	x	O	LT, GS	Questions the long term genetic stability of the GM vaccine.	Chapter 1, Section 6.4.1	As discussed in Chapter 1, Section 6.4.1 the applicant has demonstrated the stability of the vaccine through multiple passages in tissue culture. The GM vaccine has also been used in multiple clinical trails and demonstrated an acceptable safety profile. TGA has primary regulatory responsibility for assessing the quality, efficacy and patient safety of the vaccine for use in humans. TGA requires that all pharmaceuticals are required to be manufactured according to <i>Australian code of good manufacturing practice for medicinal products</i> (Therapeutic Goods Administration 2002) which includes requirements for the establishment, use and characterisation of master and seed lots in order to prevent the unwanted drift of properties which might ensue from repeated subcultures or multiple generations.
			HGT	Questions the ability of the GMO to combine with humans and other organisms	Risk scenario 4	Flaviviruses are RNA based viruses which replicate in the cytoplasm of the host cell and do not produce DNA as part of their lifecycle and therefore incorporation of viral sequences into the DNA genome in the nucleus of the cell would not be expected occur. Risk scenario 4 has been updated to expand the assessment of the potential of the GMO to recombine with humans and other animals.
			U	Questions whether there is any evidence that the GMO will pose no unforeseen risks		A large number of risk scenarios were considered in the risk assessment process and no evidence was found which lead to the identification of a risk greater than negligible posed by dealings with the GMO.
			C	Requests clarification as to whether the vaccine would be approved for people over the age of 18 years or 12 months.	Chapter 1, Section 4	The TGA has primary responsibility for assessing the risks associated with the use of the GMO as a vaccine in humans and is responsible for determining the appropriate age restriction for this vaccine. The RARMP was modified to reflect the advice provided by the TGA and to clarify that use would be permitted to people over the age 12 months. Chapter 1, Section 4
			LC	Questions why the 'persons authorised to carry out the dealings' in the licence is not limited to registered medical practitioners.	Chapter 3, Section 3.2.3	The dealings authorised by the licence are the import, transport and disposal of the GMO and as a commercial release it is considered appropriate that any person in Australia be permitted to carry out these dealings. Access to, and the use of, the GMO as a vaccine will be regulated by the TGA through the conditions of listing on the ARTG and the scheduling of the vaccine on the <i>Standard for the Uniform Scheduling of Medicines and Poisons</i> .

Sub. No:	Position	Type	Issue	Summary of issues raised	Consideration in RARMP	Comment
			A	Concerned about the suitability of the applicant to hold a licence		The Regulator has considered the suitability of the applicant to hold a licence in accordance with the relevant provisions of the Act and decided that Sanofi is suitable to hold a licence.
3	n	O	L	Has no issue with the release Vaccine should be labelled as GM for consumer information		It is intended that the vaccine will be listed as a Schedule 4 – Prescription Only Medicine. Labelling of pharmaceuticals is regulated by the TGA. TGA has advised that labels will describe the GM vaccine as a 'live, attenuated, recombinant JE virus vaccine'.