Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 148

Commercial supply of Dengvaxia, a live attenuated GM dengue vaccine

Applicant: Sanofi-Aventis Australia Pty Ltd

June 2017

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 148

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application. The licence authorises import, transport, storage and disposal of a genetically modified (GM) dengue vaccine, known as Dengvaxia, for the purpose of its commercial supply as a therapeutic product.

A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the requirements of the *Gene technology Act 2000* (the Act) and corresponding State and Territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment and no specific risk treatment measures are imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

Before Dengvaxia can be used as a therapeutic agent, Sanofi-Aventis Australia Pty Ltd (Sanofi) must also obtain regulatory approval from the Therapeutic Goods Administration (TGA). Medicines and other therapeutic goods for sale in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods (ARTG). The TGA consults the OGTR during the assessment of applications for therapeutic products that are, or contain, genetically modified organism (GMOs). Sanofi will also need approval from the Department of Agriculture and Water Resources for import of the GM vaccine.

The application

Application number	DIR 148
Applicant	Sanofi-Aventis Australia Pty Ltd
Project title	Commercial supply of Dengvaxia, a live attenuated GM dengue vaccine[1]
Parent organism	Yellow fever virus strain 17D (YF17D)
Modified trait	Altered antigen expression
Genetic modification	YF17D pre-membrane gene (*prM*) replaced with Dengue virus pre-membrane gene YF17D envelope gene (*E*) replaced with Dengue virus envelope gene
Proposed release dates	Ongoing from the date of approval
Proposed locations	Medical facilities throughout Australia including specialist travel clinics, general practitioners and those belonging to the Australia Defence Force (subject to registration by the Therapeutic Goods Administration)

[1] The title of the project as supplied by the applicant is 'Commercial distribution and prescription of Dengvaxia in Australia.'

Purpose	Import, storage, transport and disposal of the GM Dengvaxia vaccine associated with its commercial release as a therapeutic product (subject Therapeutic Goods Administration approval)

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed dealings, either in the short or long term, are negligible.

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

Credible pathways to potential harm that were considered included whether or not expression of the introduced genes and genetic modifications could alter characteristics that may impact on the disease burden from the GM vaccine strains, or produce unintended changes in viral characteristics. The opportunity for gene transfer to other organisms and its effects (if it were to occur) was also considered.

The principal reasons for the conclusion of negligible risks are that:

- exposure to Dengvaxia would be minimised by well-established clinical, import, transport, storage and disposal procedures; and
- the GM vaccine strains can survive outside of a host only for short periods, and it is susceptible to common chemical decontaminants.

Risk management plan

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

As the level of risk is considered negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions to ensure ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
ARTG	Australian Register of Therapeutic Goods
cDNA	complementary DNA
CYD	chimeric yellow fever/dengue virus (GM vaccine strain)
DAWR	Department of Agriculture and Water Resources
DENV	Dengue virus
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
PFU	plaque forming unit
prM	pre-membrane
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
TGA	Therapeutic Goods Administration
the Act	*Gene Technology Act 2000*
UV	ultraviolet
WHO	World Health Organisation
YEL-AVD	Yellow fever associated viscerotropic disease
YFV	Yellow fever virus

Chapter 1 Risk assessment context

Section 1 Background

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

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

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

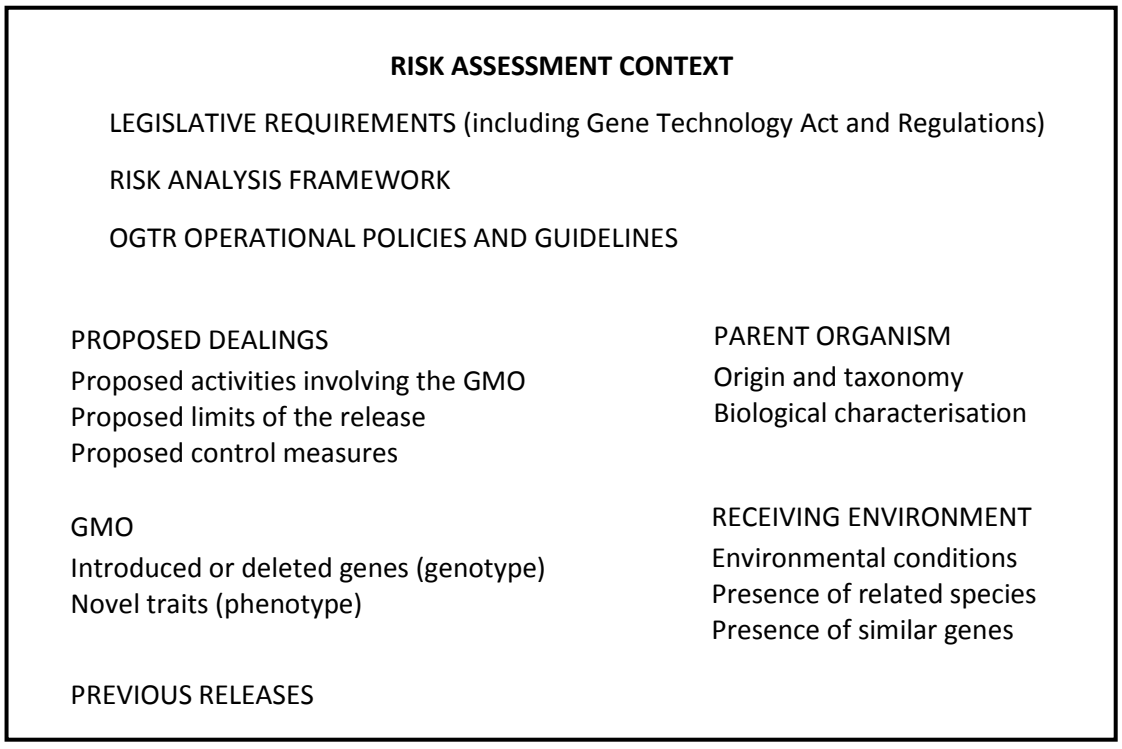

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. The Regulations and Sections 50, 50A and 51 of the Act outline the matters that the Regulator must consider and the consultation that is required when preparing a Risk Assessment and Risk Management Plan (RARMP). In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. As this application is for commercial purposes, under Section 50(3) of the Act, two rounds of consultation are required:

- In the first round, required by Section 50(3), the Regulator sought advice on matters relevant to the preparation of the RARMP from prescribed experts, agencies and authorities. Advice was sought from the Gene Technology Technical Advisory Committee (GTTAC), State and

Territory Governments, Australian Government authorities/agencies prescribed in the Regulations, all Australian local councils and the Minister for the Environment. The issues raised in their submissions are summarised in Appendix A.

- In the second round, required by Section 52 of the Act, the Regulator sought advice on the consultation RARMP from the aforementioned groups as well as the public. Advice from the prescribed experts, agencies and authorities in the second round of consultation, and how it was taken into account, is summarised in Appendix B. Seven public submissions were received and their consideration is summarised in Appendix C.

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

2.1 Interface with other regulatory schemes

7. Gene technology legislation operates in conjunction with other regulatory schemes that regulate GMOs or genetically modified (GM) products in Australia. Dealings conducted under a licence issued by the Regulator may also be regulated by the Therapeutic Goods Administration (TGA), Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Department of Agriculture and Water Resources (DAWR). Dealings may also be subject to the operation of State legislation declaring areas to be GM, GM-free, or both, for marketing purposes.

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

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

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

11. The TGA provides a national system of controls for therapeutic goods. It administers the provisions of the *Therapeutic Goods Act 1989* which specifies the standard that must be met before a vaccine can be registered on the Australian Register of Therapeutic Goods (ARTG). Inclusion in ARTG is required before a vaccine can be lawfully supplied in Australia. As part of this process, the TGA would assess the quality, safety and efficacy of the vaccine. Quality aspects could include batch-to-batch consistency in vaccine composition, purity and potency. Safety aspects could include toxicological and allergenicity profile of the vaccine, including any excipients, by-products and impurities from manufacture.

12. The administration/use of GMOs as therapeutics is not regulated under gene technology legislation. The Regulator notes that as part of the safety assessment, the TGA would evaluate viral shedding as well as risks to vaccine administrators, recipients and their carers who may be present during administration of a vaccine. The Regulator does not assess vaccine excipients and would not assess manufacturing by-products and impurities unless they are GM products.

13. The labelling, handling, sale and supply of scheduled medicines is regulated through the *Scheduling Policy Framework for Medicines and Chemicals* (AHMAC 2015). Guidelines for the safe handling, storage and distribution of Schedule 4 medicines such as vaccines are specified through the *Australian Code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011). The provisions of this Code, which ensure that quality is maintained during wholesaling, are applied

through applicable State and Territory therapeutic goods/drugs and poisons legislation, and/or State or Territory wholesaler licensing arrangements.

Section 3 Background to the DIR application

14. Sanofi-Aventis Australia Pty Ltd (Sanofi) has proposed the commercial supply of Dengvaxia, a tetravalent live attenuated GM dengue vaccine (the GMOs) in Australia. No dengue vaccine, GM or non-GM, is currently approved for use in Australia.

15. The proposed indication is the prevention of dengue caused by dengue virus serotypes 1 to 4, in individuals aged 9 to 60 years living in areas with endemic dengue. Dengvaxia is contraindicated for pregnant or breastfeeding women, individuals who are immunosuppressed, or have a history of adverse reactions to the vaccine excipients or to Dengvaxia.

16. If approved by both the Regulator and the TGA, Sanofi intends to supply Dengvaxia to specialist travel clinics, medical wholesalers, pharmacies, hospitals, general practitioners, the Department of Health and the Australia Defence Force.

17. Dengvaxia would be delivered by subcutaneous injection. The vaccine would only be available under prescription and administered as a three-dose regimen on a 0/6/12 month schedule by a suitably qualified person such as a registered medical practitioner or nurse. It would be supplied as a single dose freeze-dried powder to be reconstituted in 0.4% sodium chloride before use.

18. Australia was among the countries where Phase IIa and Phase III clinical trials for Dengvaxia (then called ChimeriVaxTM-DEN) were conducted. These trials were authorised under a licence issued by the Regulator (DNIR-386).

19. The recombinant DNA technology (ChimeriVaxTM technology) used to construct the GMOs in Dengvaxia was also used to generate the GM live attenuated Japanese encephalitis vaccine (IMOJEV). IMOJEV was approved for commercial distribution in Australia in 2010 by both the TGA and the Regulator (licence DIR 098).

20. Sanofi proposes to manufacture Dengvaxia outside of Australia.

Section 4 Proposed dealings

21. For the ongoing commercial supply of Dengvaxia, the dealings assessed by the Regulator are:

- import;
- transport;
- disposal; and

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

4.1 Details of the proposed activities

22. Dengvaxia would be imported from France. The import requires a permit from DAWR and authorisation from the TGA.

23. Dengvaxia is presented as a powder contained in a glass vial with a rubber stopper, an aluminium seal and a flip-off polypropylene cap. Single dose vials will be packaged in secure secondary cartons and 100-120 of these would be boxed in corrugated cardboard for shipping.

24. Dengvaxia would be distributed by a commercial courier that specialises in the handling of temperature sensitive pharmaceuticals and vaccines, and would be licenced by the TGA for wholesale storage and packaging of these items. Prior to distribution, Dengvaxia would be stored in warehouse cool rooms (2-8°C) where access would be limited to authorised personnel.

25. Material exposed to Dengvaxia would be decontaminated by a method approved by the Environmental Protection Agency of each State/Territory. Dengvaxia disposed from the warehouse, distribution end point or vaccination facilities would be inactivated by a clinical waste disposal method, such as high temperature incineration. At the warehouse, expired vaccine would be disposed of by a clinical waste contractor and all destroyed stock would be accounted for. At the medical facilities where vaccination occurs, residual vaccine and waste associated with the vaccination process would be disposed of in the clinical waste stream.

26. Disposal of biohazardous material would be in accordance with the requirements of the Work Health and Safety Act 2011 and related state and territory legislation. Secondary and tertiary packaging, which would not usually be contaminated with Dengvaxia, will be disposed of in normal waste streams.

27. Storage, handling and transport would be in accordance with the WHO's Good distribution practices for pharmaceutical products (WHO 2010).

Section 5 Parent organisms

28. Each of the four GMOs in Dengvaxia has been constructed from genes derived from the yellow fever virus (YFV) and one dengue virus (DENV) serotype. There are four distinct DENV serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), which share 60-75% identity at the amino acid level (Guzman & Harris 2015). To provide a risk context, this Section contains background information on the two parent organisms, YFV and DENV. They are very closely related arboviruses (arthropod borne viruses) that are transmitted by female *Aedes* mosquitoes. They both belong to the genus Flavivirus in the *Flaviviridae* family and are classified as Risk Group 2 organisms (Standards Australia & Standards New Zealand 2010).

5.1 Pathology

29. Yellow fever and dengue are self-limiting, flu-like illnesses that share many symptoms with other flavivirus infections and for that reason, are commonly misdiagnosed. Dengue, in particular, is often confused with other mosquito-borne diseases such as Chikungunya and Zika. The majority of YFV and primary DENV infections show no or few symptoms (CDC 2015a). The severe form of both diseases is biphasic with an acute febrile phase followed by a toxic phase with a haemmorhagic fever (World Health Organisation 2012).

30. YFV and DENV differ in their tropism. YFV is neurotropic (proliferates in nervous tissue) as well as viscerotropic (proliferates in multiple internal organs) while DENV is primarily considered a non-neurotropic virus although it can cause encephalitis.

5.1.1 Yellow fever

31. For individuals who develop disease, the acute phase begins 3-6 days after being bitten by an infected mosquito. It is marked by the sudden onset of a very high fever (CDC 2015b) that lasts about four days. The fever may be accompanied by other non-specific symptoms such as chills, headache, myalgia, back pain, anorexia, nausea, vomiting and tiredness. During this stage, the patient is sufficiently viremic to infect feeding mosquitoes (Monath et al. 2008).

32. Most cases of mild yellow fever resolve after 2-5 days of symptoms with no lasting sequelae. The fever falls abruptly and symptoms abate, corresponding to viral clearance from the blood and the production of YFV-specific antibodies. In 15-25% of yellow fever infections, this remission lasts only about a day before patients enter the toxic phase.

33. Characteristics of the toxic stage are excessive amounts of albumin in the urine, "black vomit" and the characteristic jaundice which gives yellow fever its name. These symptoms indicate kidney failure, gastrointestinal haemorrhaging and liver failure, respectively (WHO 2017b). 20-50% of the patients who enter the toxic phase die 10-14 days from the onset of disease (Lefeuvre et al. 2004).

34. The terminal events in yellow fever are cytokine mediated as the patient is no longer viremic even though YFV antibodies are present (Burke & Monath 2001; Monath et al. 2008).

35. There is no specific antiviral medication for yellow fever. Treatment usually entails ameliorating the fever and myalgia can be ameliorated with analgesics. A highly effective prophylactic vaccine has been available for several decades, as described in Chapter 1 Section 5.6.

36. Recovery from yellow fever leaves no lasting organ damage and confers lifetime immunity to YFV.

5.1.2 Dengue

37. The WHO classifies dengue severity in three categories: dengue without warning signs, dengue with warning signs, and severe dengue (Figure 2) (World Health Organisation 2009). About 75% of dengue infections are subclinical. Dengue presents as a sudden onset of a high fever (40°C) accompanied by two of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms appear after an incubation period of 4-10 days, last for 2–7 days and are usually followed by a recovery phase.

38. Dengue infections can deteriorate into a potentially lethal form called severe dengue. As shown in Figure 2, there may or may not be signs warning of the impending decline in health. These signs are listed in Figure 2. Severe dengue can manifest as a haemorrhagic fever or dengue shock syndrome (DSS). With the latter, patients enter a state of hypovolemic shock as severe plasma leakage prevents the heart from pumping enough blood through the body. Patients who enter this state can die within 12-36 hours (WHO 2017a; Public Health Agency of Canada 2014).

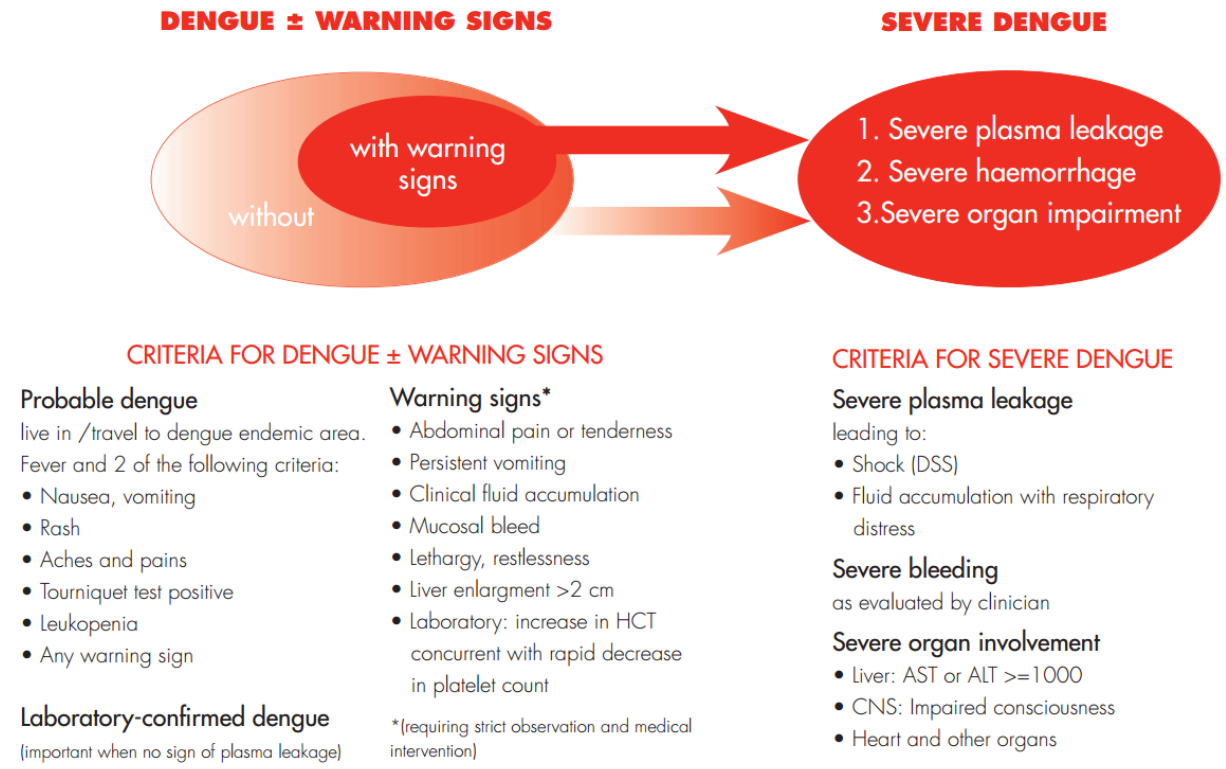

Figure 2. Dengue classification and level of severity

39. No specific antivirals exist to treat dengue. Judicious medical care and fluid replacement can reduce the fatality rate of severe dengue from 20% to 1% (Simmons et al. 2015). Mild symptoms are managed with analgesics.

40. Recovery from primary DENV infection confers lifelong immunity to the same serotype (homologous serotype) and cross-protection against a different serotype (heterologous serotype) for only about two years after infection (WHO 2017a).

41. The 50% human infective dose for DENV is less than 10 PFU (Blaney, Jr. et al. 2010).

5.1.3 Secondary dengue infections

42. The overall risk of severe dengue is low (a few per cent of symptomatic cases). Severe dengue can occur in primary infections but is rare. Most cases of severe dengue occur in secondary infections, particularly in individuals who have previously been exposed to a different DENV serotype. Age also has an impact on disease severity, with secondary infection being a greater risk factor in children than adults (Guilarde et al. 2008; Hammond et al. 2005). Severe dengue can be caused by all DENV serotypes.

43. A large proportion of DENV-specific antibodies are cross-reactive with all DENV serotypes and with other closely related flaviviruses (Henchal et al. 1982). They are, however, not cross-protective against these same serotypes and viruses.

44. The pathogenesis of severe dengue is only partially understood and may be caused by antibody dependent enhancement (ADE). DENV is predominantly a lymphotrophic agent and the principal target cells for DENV replication are mononuclear phagocytes. In severe dengue, pre-existing antibodies from an earlier infection bind the invading serotype with poor avidity. These cross-reactive antibodies cannot neutralise the different serotype but can form an antibody-DENV complex which attaches to circulating immune cells. This facilitates the infection of these cells with DENV, promotes viral replication and increases the risk of severe dengue (Whitehead et al. 2007).

45. The severity of a second infection is attributed to an immunopathological reaction rather than to the virus itself. When DENV is not neutralised by pre-existing antibodies, cytokines are released. At the onset of severe dengue, cytokines are at or near their peak levels and viremia is declining steeply. Some cytokines increase capillary permeability transiently and cause the haemorrhaging or hypovolemic shock that characterises severe dengue.

46. Infants aged between 6-12 months are proposed to be at greatest risk of developing a severe primary dengue infection if they are born to mothers with previous dengue exposure. The passively acquired maternal DENV antibodies, which are waning and at sub-neutralizing levels, could enhance the disease although some authors were not able to establish a link between enhancing antibodies in infant's sera and disease outcome (Burke & Monath 2001; Wahala & Silva 2011; Libraty et al. 2009).

47. Infection with DENV for a third or fourth time is less severe than the second infection (Olkowski et al. 2013; Gibbons et al. 2007).

5.1.4 Co-infection

48. Individuals can be co-infected with two different DENV serotypes (Figueiredo et al. 2011) as well as multiple genotypes of a single serotype (Aaskov et al. 2007).

49. The frequency of recombination in Flaviviruses is extremely low (Taucher et al. 2010) but recombination has been observed between different DENV genotypes but not between different flaviviruses (Twiddy & Holmes 2003; Aaskov et al. 2007; Tolou et al. 2001; Worobey et al. 1999). In a patient co-infected with both DENV and Zika virus, there was no evidence of viral recombination as determined by real-time RT- PCR (Dupont-Rouzeyrol et al. 2015). Co-infections of cell lines with YFV constructs that were specifically designed to strongly favour recombination did not generate new chimeric viruses (McGee et al. 2011).

50. In humans co-infected with DENV and Zika virus or with DENV and Chikungunya virus (CHIKV), there is some degree of viral interference but little or no synergistic effects between the two viruses with regards pathology of disease (Dupont-Rouzeyrol et al. 2015; Chang et al. 2010; Salas-Benito & De

Nova-Ocampo 2015). Like DENV and YFV, Zika virus and CHIKV are both transmitted by *Aedes* mosquitoes.

51. Mosquitoes can be co-infected with at least three DENV serotypes (Thavara et al. 2006) and mosquito cell lines can be co-infected with at least three different types of viruses (Kanthong et al. 2010).

5.2 Epidemiology

5.2.1 Pattern of distribution

52. As DENV and YFV are mosquito vectored, the countries at greatest risk of dengue and yellow fever lie in the wet tropical and sub-tropical regions of the world. 40% of the world's population live in these areas.

53. YFV is well established in sub-Saharan Africa, Central and South America. The WHO estimates that over 90% of YF cases occur in Africa, with the majority of deaths occurring in West Africa. During urban epidemics in Africa, up to 20% of the population manifest symptoms and 40% are seropositive (Wasserman et al. 2016). Despite the presence of suitable vectors in Asia, yellow fever was not reported until 2016 when multiple cases of yellow fever resulted from infected individuals returning to China from Angola.

54. Dengue is more widespread than yellow fever and has increased 30-fold in the past 50 years, making it the most rapidly spreading mosquito-borne disease. It is present in most warm, non-arid regions of the world (Figure 3) and the WHO estimates 50-100 million people are infected with DENV each year (WHO 2017a). Dengue is hyperendemic (constantly present at a high rate) in Asia and Latin America, and severe dengue is the leading cause of illness and deaths in children living in these areas (Murray et al. 2013). Africa is less severely affected.

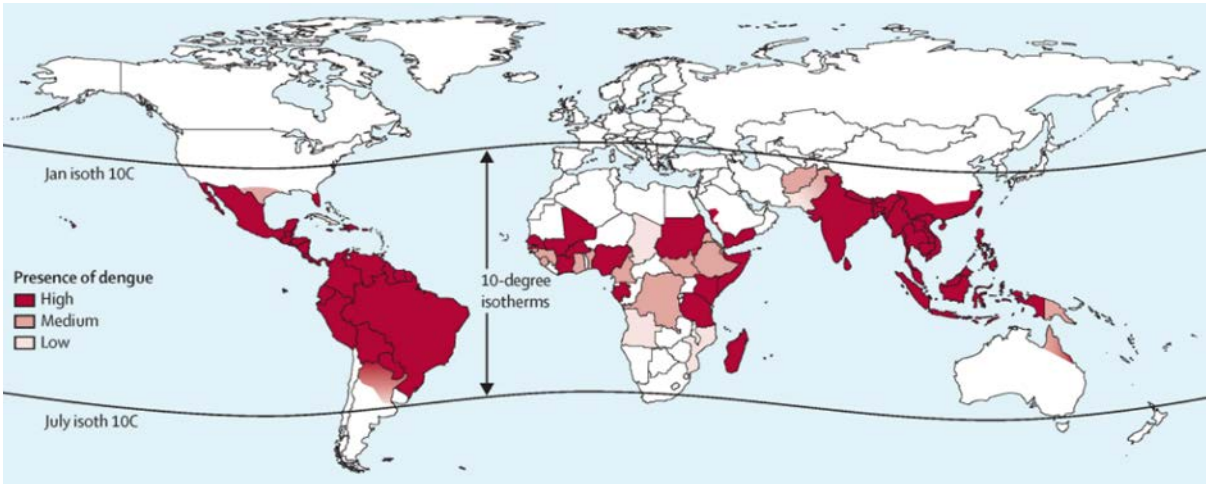

Figure 3. Regions at risk of dengue

5.2.2 Outbreaks in Australia

55. Dengue is not endemic in Australia but can be acquired overseas and brought into Australia (Department of Health 2016). This has resulted in sporadic outbreaks as one of the mosquito vector species, *Aedes aegypti*, is endemic in northern Australia. The largest recent outbreak occurred in Cairns between November 2008 and May 2009, and resulted in 915 confirmed cases, including six cases of severe dengue and one death (Ritchie 2013). To date, there have been no reports of yellow fever in Australia.

5.2.3 Ecology and reservoirs

56. YFV and DENV can be maintained in a sylvatic cycle or an urban cycle. In the sylvatic or jungle cycle, mosquitoes transmit the viruses between non-human primates while in the urban cycle mosquitoes transmit the viruses between humans. The viruses transfer from the sylvatic to the urban cycle when infected mosquitoes opportunistically feed on humans who enter the jungle.

57. YFV is primarily a sylvatic virus affecting small primates and it occasionally transmits to humans. The zoonosis results in an epidemic that tends to be self-limiting due to the high human fatality rate. In both South America and Africa where YFV is endemic, non-human primates serve as a reservoir for YFV.

58. YFV can also infect many mammals without pathological outcomes. It has been detected in sloths and rodents (Laemmert 1948; de Thoisy et al. 2004) but they are not classified as hosts as the maximum level of viremia is below transmission thresholds. Antibodies to YFV have also been detected in domesticated animals such as cattle, sheep and goats (Adu et al. 1990). Animals used to study YFV include mice (Meier et al. 2009), hamsters (Tesh et al. 2001; Xiao et al. 2001) and rhesus monkeys (Monath et al. 1981).

59. Humans are the main vertebrate hosts of DENV and it is hyperendemic in many densely populated countries. DENV is self-sustaining in many urban areas as it is less virulent than YFV and *Ae. aegypti* thrives in an urban environment. The DENV sylvatic cycle generally involves the smaller monkeys (Vasilakis et al. 2011; Althouse et al. 2012) although DENV antibodies have been detected in orangutans, South American rodents, marsupials and bats (de Thoisy et al. 2004; Wolfe et al. 2001).

60. The lack of suitable non-human primate hosts outside captivity would preclude a sylvatic cycle being established for DENV and YFV in Australia.

61. *Ae. aegypti* and *Ae. albopictus* eggs can undergo diapause or suspended development during the dry season and potentially serve as a second reservoir for these viruses. *Ae. aegypti* eggs are more tolerant of exposure to colder, drier conditions so it has the potential to spread disease into regions which have mild winters or are drier (Lacour et al. 2015; Thomas et al. 2012).

5.2.4 Transmission

62. YFV and DENV are transmitted by *Aedes* mosquitoes. *Ae. aegypti* (also called yellow fever mosquito) is the most capable vector but *Ae. albopictus* (also called the Asian tiger mosquito) is the more common vector in South East Asia. In Australia, *Ae. aegypti* is found in northern Queensland, the Northern Territory and northern Western Australia while *Ae. albopictus* is present in the Torres Strait Islands. Other mosquito vectors include *Ae. polynesinesis* and *Ae. scutellaris*, which are common in many south Pacific islands and in New Guinea, respectively. YFV and DENV are also spread by *Haemagogus* and *Sabethes* mosquitoes (both tree-hole breeding mosquitoes) in Central and South America.

63. Infected female mosquitoes transmit viruses during feeding when they inject their saliva into their prey. The saliva contains a mild anaesthetic and anti-coagulants to facilitate feeding (Ribeiro et al. 2001; Foster & Walker 2009). The blood meal is required to produce viable eggs.

64. To be successfully transmitted by a mosquito, a virus must survive ingestion, replicate and infect the mosquito's salivary glands. For DENV, *Ae. albopictus* is a less effective vector than *Ae. aegypti* because even though *Ae. albopictus* is more easily infected with DENV, its saliva is less infectious (Lambrechts et al. 2010; Whitehorn et al. 2015; Vasilakis et al. 2011).

65. Mosquitoes feed for a very short time and are most likely to be infected when feeding on a highly viremic individual. This ensures that enough virus is ingested to exceed the midgut infection threshold. Estimates of viral titre required for infection range from 10^5 to $10^{7.5}$/ml (Burke & Monath 2001; Nguyet et al. 2013). DENV viremia is highest on days 3-7 of illness (Tomashek et al. 2016) while

YFV viremia is highest on days 3-5 of illness (Gershman & Staples 2015). Viremic individuals without significant symptoms can still infect mosquitoes during this period.

66. Once infected with a virus, a mosquito can transmit that virus for the rest of its life. The lifespan of an *Aedes* mosquito is about three weeks. To persist in a mosquito, viral replication must be balanced with the mosquito's antiviral response so that the virus is neither cleared nor disadvantages the mosquito (CDC 2012). The period between ingestion of the virus and the mosquito becoming infectious is 12-21 days for YFV and 4-10 days for DENV (WHO 2017a), depending on the ambient temperature (Chan & Johansson 2012; WHO 2017a).

67. YFV and DENV can be transmitted vertically. In humans, an infected mother can transfer DENV to her foetus (Phongsamart et al. 2008). In mosquitoes, females can transmit viruses during egg laying if the genital tract is infected (Espinosa et al. 2014; Buckner et al. 2013; Fontenille et al. 1997) and male progeny that hatch from theses eggs can transmit the viruses horizontally during copulation (Mavale et al. 2010).

68. YFV and DENV can be transmitted in the absence of a mosquito but this is rare. Dengue has resulted from transfusion of blood from an infected donor (Tambyah et al. 2008; Chuang et al. 2008), organ transplants (Saigal et al. 2013; Tan et al. 2005), needlestick injuries by laboratory and healthcare workers (Langgartner et al. 2002; Ohnishi 2015; Lee et al. 2016), and mucous membrane contact with infected blood (Chen & Wilson 2004). Successful non-vector transmission increases with the viral concentration in the inoculum and the volume transferred.

5.2.5 Control, environmental stability and decontamination methods

69. The primary method for preventing the spread of DENV and YFV is by controlling mosquito populations. The yellow fever vaccine (see Chapter 1 Section 5.6) is highly effective but yellow fever has not been eradicated due to the sylvatic reservoir and complacency with vaccination between outbreaks.

70. DENV and YFV cannot survive for extended periods outside the host or vector. DENV are stable in dried blood for up to nine days at room temperature (Public Health Agency of Canada 2014). YFV is more fragile, with 0.16% or less remaining viable after 60 minutes when aerosolised at 27°C with a relative humidity of 30-80% (Mayhew et al. 1968).

71. Due to their membrane envelopes, flaviviruses can be chemically inactivated by low pH, detergents, disinfectants and chaotropic agents. DENV is susceptible to 1% sodium hypochlorite, 2% gluteraldehyde, 2% peracetic acid, 70 % ethanol, iodophors, phenolic compounds, and 3-6% hydrogen peroxide (Public Health Agency of Canada 2014).

72. Flaviviruses can be physically inactivated by ultraviolet light, desiccation, γ-irradiation and heat. YFV is heat inactivated after 30 minutes at 60°C (Canadian Office of Laboratory Security 2001). DENV is sensitive to moist heat (121°C for at least 15 min), dry heat (160-170°C for at least 1 hour), and low temperature sterilization (ethylene oxide or plasma sterilization) (Public Health Agency of Canada 2014).

5.3 Structure and genomic organisation

73. The genomes of YFV and DENV are similarly organised. They comprise a single 11 kb strand of positive-sense RNA which doubles as the mRNA. The mRNA has a single open reading frame flanked by short untranslated regions.

74. The genome is translated into a single polypeptide. This is post-translationally cleaved by viral and host proteases into 10 proteins (Ruiz-Linares et al. 1989) which are identically ordered and have analogous functions in both YFV and DENV. The three structural proteins are grouped on the N-terminal portion as shown in Figure 4.

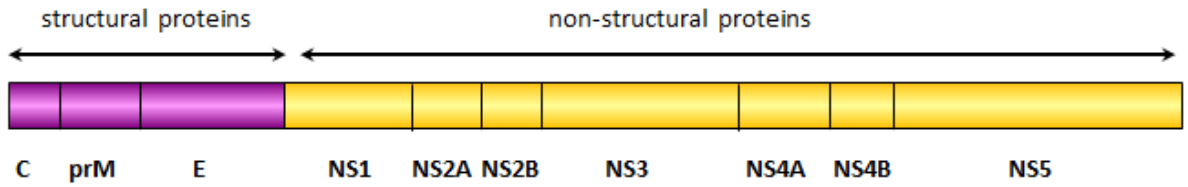

Figure 4. Organisation of the proteins in YFV and DENV

75. The seven non-structural proteins are primarily replicative proteins although some are also involved with confounding the host immune system (Munoz-Jordan et al. 2005). The proteins, as ordered from N-terminus of the polyprotein, are the:

- capsid protein (C)
- pre-membrane protein (prM), a glycoprotein which is cleaved by cellular proteases after viral particle assembly to produce the mature membrane protein (M)
- envelope protein (E), a glycoprotein which binds the cellular receptor, and mediates fusion of viral and host membranes
- non-structural protein NS1 which, in association with NS4a, is required for RNA replicase function
- non-structural protein NS2A which is involved in RNA replication and viral assembly
- non-structural protein NS2B which, in association with NS3, cleaves the polyprotein
- non-structural protein NS3 is a serine protease on its N-terminus and an RNA helicase on its C-terminus. The protease moiety requires NS2B for catalytic activity (Noble et al. 2012).
- non-structural protein NS4A which, in association with NS1, is required for RNA replicase function and viral assembly
- non-structural protein NS4B which is involved in RNA replication, viral assembly
- non-structural protein NS5 which has RNA-dependant RNA polymerase and methyl transferase activity.

76. YFV and DENV are enveloped viruses, meaning a lipid bilayer surrounds the capsid containing the viral genomic RNA. Attached to the viral envelope and exposed to the exterior are the membrane (M) and envelope proteins (E).

5.4 Membrane protein (M) and envelope protein (E)

77. prM, M and E are the main antigenic determinants on both YFV and DENV. There are 180 copies of both M and E on the viral surface but as E (50 kD) is significantly larger than M (9 kD), E is the primary target of neutralising antibodies.

78. M is expressed as a precursor (prM). The virion is not infectious if prM is not cleaved into pr and M by host proteases as E does not enter its pre-fusion conformation. prM facilitates virus particle assembly and proper folding of E to produce mature virions. Prior to viral exit from the cell, the majority of prM is cleaved by a furin-like cellular protease to produce the mature M on the surface of the viral particles. The released 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).

79. E determines the cellular tropism and hosts that could be susceptible to the viruses. It binds the host receptor, and facilitates fusion of the viral and host membranes. The host receptors bound by YFV and DENV have not been identified with certainty. After the virus binds the cell, it is endocytosed and targeted for destruction by the host cell. As part of this process, the endosome is acidified and the ensuing pH drop triggers a conformational change in all 180 copies of the E protein, enabling them to attach to the host membrane. This results in a concerted distortion of the host membrane which

induces fusion of viral and host-cell membranes, releasing the genome into the cytoplasm where replication takes place (Patkar et al. 2007; Smit et al. 2011).

80. E has several oligomeric states. In immature virions E exists as a trimer of heterodimers with prM, while in mature virions E exists as a metastable dimer in its pre-fusion conformation. During the pH triggered structural rearrangements, the dimer dissociates into monomers, which form trimers during membrane fusion (Li et al. 2008; Smit et al. 2011; Modis et al. 2004; Stiasny et al. 2009).

81. prM and E must be co-expressed as a major function of M is to prevent the pre-mature structural rearrangement of E into an oligomeric form that would preclude infectivity. (Yu et al. 2009; Knipe & Howley 2007). The sensitivity of these proteins to pH, proteolytic and lipolytic enzymes prevents Dengue virus infection by oral routes in humans.

5.5 Replication

82. In humans, YFV infects cells of multiple organs while DENV primarily infects cells of the myeloid lineage such as macrophages, monocytes and dendritic cells. It is released from these cells into the bloodstream which spreads it to peripheral organs. DENV can also infect hepatocytes and endothelial cells.

83. Replication does not involve a DNA intermediate. The positive-sense genomic RNA serves as both the template for negative-strand RNA synthesis and mRNA. As in other flaviviruses, strand synthesis is asymmetric and the positive (genomic) strand accumulates 10 times as fast as negative strand (Gong et al. 1996; Cleaves et al. 1981). Replication occurs in the cytoplasm.

84. The newly synthesized viral RNA is enclosed by the capsid which then gains the viral envelope from the rough endoplasmic reticulum (Ruiz-Linares et al. 1989). The assembled particle travels through the Golgi apparatus complex where prM is cleaved, converting the virions into their mature, infectious form. The virions are transported to the cell surface via intracellular vesicles where they are released from the cell through exocytosis (Stiasny & Heinz 2006; reviewed in Mukhopadhyay et al. 2005).

5.6 YF 17D, the Yellow fever vaccine strain

85. YF 17D is the current live yellow fever vaccine strain (marketed as YF-VAX® or Stamaril® by Sanofi-Pasteur). It confers lasting immunity against yellow fever and has a long history of safe use (FDA 2016). YF 17D was originally derived from virulent wild-type YFV by Max Theiler in 1935. To derive an attenuated YFV, Theiler serially passaged the Asibi isolate 18 times in mouse embryo tissue, 58 times in minced whole chick embryos and 128 times in chicken embryonic tissue with the spinal cord removed (Theiler & Smith 1937; Smith & Theiler 1937). During one of the passages in chicken embryonic cells without nervous tissue, the viscerotropic and neurotropic properties were lost. The vaccine is currently manufactured from either substrain YF17D DD or YF 17D-204, which arose from passages 195 and 204, respectively. These two substrains share 99.9% sequence homology (Pugachev et al. 2002).

86. YF 17D is not virulent and is attenuated in its ability to replicate. As compared to wild-type YFV, YF 17D is rarely neurotropic or viscerotropic (WHO 2017b). YF 17D was first tested as a yellow fever vaccine in humans in 1937. Its subsequent widespread use has significantly decreased the incidence of yellow fever.

87. Vaccination with YF 17D is typically asymptomatic or causes mild symptoms such as headache, myalgia and low grade fever and soreness at the injection site (Lindsey et al. 2008). It induces a brief, self-limiting infection characterised by low level viremia, an interferon response and rapid induction of neutralising antibodies. Exposure to YF 17D is rarely associated with neurotropic and viscerotropic disease, unlike exposure to wild-type YFV (WHO 2017b).

88. Despite about half a billion vaccinations with YF 17D, very few severe adverse events (SAEs) have been reported. The SAEs include Yellow fever vaccine-associated viscerotropic disease (YEL-AVD), Yellow fever vaccine-associated neurotropic disease (YEL-AND), post-vaccinal encephalitis and severe allergic reactions to excipients (Lindsey et al. 2008; Pulendran et al. 2008; Kitchener 2004; Marfin et al. 2005). YEL-AND and YEL-AVD occur in approximately 8 and 4 per million vaccinations, respectively (CDC 2015c). Most people with YEL-AND recover completely while YEL-AVD has a 60% fatality rate. Incidence data for encephalitis is not available.

89. The SAEs have not been attributed to mutations in YF 17D. Several vaccine bulk lots, representing a 12-year manufacturing period, were sequenced. Four silent mutations were identified implying that YF 17D has not reverted to the wild-type pathogenic strain (Barban et al. 2007; Galler et al. 2001). SAEs associated with YF 17D vaccines could be attributed to pre-existing genetic susceptibility. High risk individuals are those over 65 years old, the immunocompromised and those with a history of thymus disease (Gerasimon & Lowry 2005; Vellozzi et al. 2006; Silva et al. 2009; Muñoz et al. 2008). YEL-AND most commonly affects infants, and this has led to the recommendation that YF 17D not be administered to children under the age of 9 months (Cetron et al. 2002).

5.6.1 Basis of attenuation of YF 17D

90. The development of YF 17D predates the modern molecular biology era by several decades. Due to the long lasting immune response it induces, its high efficacy and stability, YF 17D has not needed to be improved since it was derived about 80 years ago. It is not widely researched and published information on many aspects of YF 17D, as analysed with newer techniques, is lacking.

91. The precise basis for the attenuation of YF 17D is not understood. The population of YF 17D-204 is homogenous. By contrast, the Asibi isolate is a collective name for multiple YFVs isolated in 1927 from a yellow fever patient named Asibi and were the first human viruses ever isolated. As with other clinical isolates, the Asibi isolate is a highly diverse quasispecies with significant variation in its genes (Beck et al. 2014). The diversity in its subpopulations may offer the cooperative advantage that often makes clinical isolates more virulent and pathogenic. (Beck et al. 2014; Vignuzzi et al. 2006; Tangy & Despres 2014).

92. When the Asibi isolate is compared to YF 17D-204, in excess of half the amino acid changes lie in the structural genes prM and E. Due to the role of the E protein in attachment and entry to cells, the changes in E would be largely responsible for the observed attenuation in YF 17D. Some of the differences map to the domain that determines tropism and cell attachment, and these may decrease the neurotropism or viscerotropism of YF 17D (Monath et al. 2008). Since the Asibi isolate is not homogenous, the exact change in E as well as the other proteins differed in various sequence comparisons (Hahn et al. 1987; Galler et al. 1997; Beck et al. 2014).

Section 6 GM vaccine viruses in Dengvaxia – nature and effect of genetic modifications

93. YF 17D is used as a vaccine vector to deliver DENV antigens that induce an appropriate and lasting immune response without the virulence that may be associated with live DENV. The genetic modifications are designed to create non-pathogenic, replication-competent viruses that would elicit a protective immune response against the four DENV serotypes. Dengvaxia comprises four GM vaccines strains, each of these comprising most of the YF 17 D genome but with the YF 17D prM and E genes replaced by those from one of four DENV serotypes (Figure 5).

6.1 The genetic modification

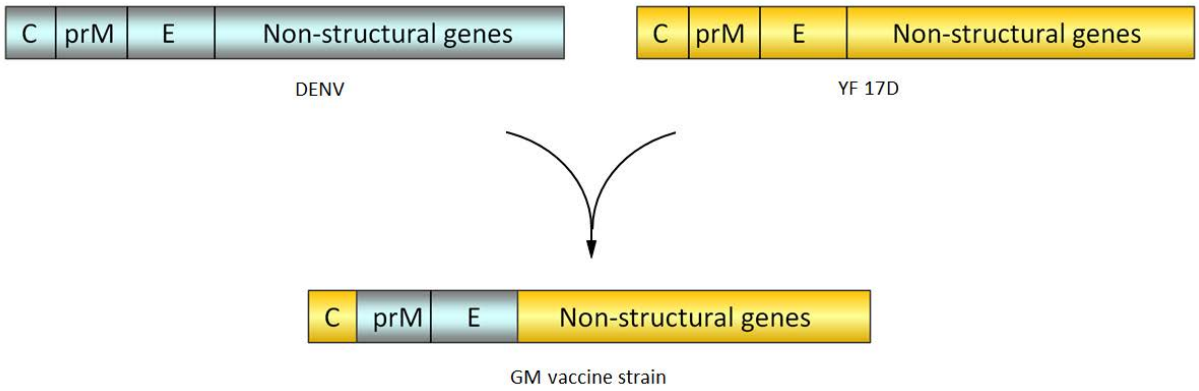

Figure 5. Construction of the GM vaccine strains

prM and E from DENV (blue) replaces the homologous genes in YF 17D (yellow). The process is repeated for all four DENV serotypes and the four resulting GMOs are present in Dengvaxia.

94. Most of the antigenic determinants of DENV lie on the E protein but E and prM must be co-expressed to yield a functional E protein that is both correctly folded and in the correct oligomeric structure (see Chapter 1 Section5.4). The proteins of flaviviruses are unusual in being expressed as a single polyprotein which is post-translationally cleaved. As YF 17D and DENV are closely related flaviviruses, expression of DENV genes in YF 17D increases the likelihood of the correction posttranslational processing of the introduced genes from DENV.

95. prM and E genes used to construct the GMOs are derived from clinical isolates of DENV that have been passaged several times in mosquito or monkey cell lines. Table 1 shows the starting isolates and the cells lines in which they were passaged (Barban et al. 2012; Guirakhoo et al. 2001; Theiler & Smith 1937).

Table 1: Derivation of YFV and DENV strains used to construct the GM vaccine strains in Dengvaxia

Virus	Strain	Starting clinical isolate from	Subsequent passages
YFD	17D (substrain 204)	Yellow fever patient Ghana 1927	18 x minced mouse embryo with 10% monkey serum 58 x minced whole chick embryo 160 x minced chicken embryo with brain and spinal cord removed
DENV-1	PUO-359/TVP-1140	Dengue fever patient Thailand 1980	2 x C6/36 cells 2 x Vero cells
DENV-2	PUO-218	Dengue fever patient Thailand 1980	1 x mosquito* 1 x LLC-MK2 1 x C6/36 cells 2 x Vero cells
DENV-3	PaH881/88	Dengue fever patient Thailand 1988	1 x AP-61 1 x C6/36 cells 2 x Vero cells
DENV-4	1228 strain (TVP-980)	Dengue fever patient Indonesia 1978	2 x mosquito* 2 x C6/36 cells 2 x Vero cells

(Guirakhoo et al. 2000; Guirakhoo et al. 2001)

Origins of the cell lines are as follows: AP-61 from *Ae. pseudoscutellaris;* C6/36 from *Ae. albopictus*; LLC-MK2 from Rhesus monkey kidney epithelia; Vero from African green monkey kidney epithelia.

**Toxorhynchites splendens* (elephant mosquito)

96. The remaining eight genes are from YF 17D substrain 204, one of the two substrains currently used to manufacture yellow fever vaccines (Stock et al. 2012). They encode the genes for replication and the capsid. All untranslated regions are also derived from YF 17D.

97. As both DENV and YFV are RNA viruses, the GMOs were constructed by reverse genetics. cDNA of the YF 17D-204 infectious clone and cDNA encoding prM/E from each DENV serotype were cloned onto separate plasmids. From these, chimeras were constructed, the resultant constructs amplified in *E. coli* and sequenced before they were transcribed using SP6 RNA polymerase to generate positive-sense infectious RNA. This was transfected into and amplified in Vero cells.

98. The DNA plasmids are only used in the construction of the GMOs and are not present in the final vaccine product.

6.2 Effect of the genetic modification

99. The GM vaccine viral particle would display the DENV M and E glycoproteins on the viral surface. These two glycoproteins would be anchored to the membrane envelope that surrounds the YF 17D capsid.

100. Each GM vaccine strain would have the antigenicity of DENV since prM and E, which carry the surface antigens, are derived from a DENV serotype. E also determines host range and tropism, and since this is derived from one of the DENV serotypes the GM vaccine strains are not expected to be neurotropic.

101. The GM vaccine strain would have the replicative traits of YF 17D since all the replicative proteins, such as the RNA polymerase, are derived from YF 17D.

6.3 Characterisation of the GMOs

6.3.1 Genotype and phenotype

102. The GM vaccine strains are genetically and phenotypically stable, non-hepatotropic and less neurovirulent than YF 17D. As compared to both parent organisms, the GM vaccine strains are more attenuated and do not replicate as well (Monath et al. 2015). YF 17D is an attenuated strain largely due to the changes accrued in the E protein. When the GM vaccine strains were constructed, these attenuating changes in YF 17D were not carried through to the vaccine strains, as E from YF 17D was replaced with the homologous protein from DENV. However, the GM vaccines strains are attenuated when tested *in vivo*, possibly because polyprotein is chimeric. Given prM and E proteins from DENV have not co-evolved with the rest of the proteins from YF 17D, less than optimal interactions between the various viral proteins may be sufficient to attenuate the chimeric viruses.

103. The GM vaccine strains are not transmitted by mosquitoes (McGee et al. 2008a; McGee et al. 2011; McGee et al. 2008b) as they cause short-lived viremia, low-level in human vaccinees (Guy et al. 2010b).

104. The GM vaccine strains were not found to be toxic in mammalian hosts when characterised in model animals and in human clinical trials (Monath et al. 2002; Monath et al. 2015; Monath et al. 2003).

6.3.2 Genotype stability

105. In this Section, the four GM vaccine strains representing the four DENV serotypes are individually referred to as CYD1 (chimera YFV DENV-1), CYD2, CYD3 and CYD4.

106. Eight of the 10 genes in the CYDs are derived from YF 17D, which has a long history of safe use as a vaccine and no record of reversion to the wild-type phenotype (Galler et al. 2001; Barban et al. 2007; Domingo & Niedrig 2009; Hahn et al. 1987).

107. Vaccine strains must be genetically stable throughout production and after vaccination to ensure a constant, safe phenotype. In the natural environment, DENV and YFV are restrained from mutating at a rate similar to other RNA viruses as they must maintain the capacity to replicate in two phylogenetically diverse hosts: primates and mosquitoes. This constraint is absent during vaccine production.

108. All four CYDs were relatively stable *in vitro*:

- In scaling-up from the pre-master seed lot to the corresponding bulk lots for clinical trials (Mantel et al. 2011), no nucleotide substitutions were detected. The method used for testing could detect changes when they constituted over 10% of the virus population.
- In scaling-up to 180L industrial production, there were no changes in CYD1, CYD2 or CYD4, and two changes in CYD3 (described in paragraph 110) which had a combined effect of being less neurovirulent than YF 17D.

109. All four CYD were assessed *in vivo* and observed to be stable:

- In samples taken from vaccinated monkeys on the last day of viremia, there were no changes in CYD2 or CYD4, one change in CYD3 and two changes in CYD1. Both the altered CYD1 and CYD3 grew to significantly lower titres than YF 17D when tested in a human cell line (HepG2) and to a similar level to YF 17D when intrathoracically inoculated into mosquitoes. The altered CYD1 was slightly more virulent than the starting CYD1 but still attenuated as compared to YF 17D (Guirakhoo et al. 2001).

- In humans, post-vaccination CYD titres were too low to ascertain definite fidelity during replication but no changes were detected in the sections that were sequenced (Morrison et al. 2010; Poo et al. 2011).
- In mosquitoes, no changes were observed in the prM and E genes 14 days after intra-thoracic inoculation (Johnson et al. 2004).

110. When the individual CYD changes were analysed, they were not found to be deleterious:

- A single change in the E protein recurred in analogous positions in CYD3 *in vitro* and in both CYD1 and CYD3 *in vivo*. In terms of neurovirulence, the change was shown to be inconsequential when tested in suckling mice (Guirakhoo et al. 2004) and resulted in reduced viremia when the CYD was subcutaneously inoculated into monkeys. This change may reflect an adaption to growth in a monkey cell line. The DENV strains used to construct the four CYDs were isolated from humans and only passaged twice in Vero cells (see Table 1), which are of monkey origin. During industrial production and pre-clinical *in vivo* studies, the CYDs were grown for multiple cycles in monkey cells or monkeys.
- A change was also observed in NS4B of CYD3. An analogous change in CYD1 was found to decrease neurovirulence and viscerotropism in suckling mice and monkeys, respectively (Guirakhoo et al. 2004).

6.3.3 Transmission by mosquitoes

111. The CYDs are not transmitted by mosquitoes after feeding (Higgs et al. 2006). Two weeks after an artificial blood meal loaded with CYD2, 16% of *Ae. aegypti* and 24% of *Ae. albopictus* were infected but the virus did not disseminate to mosquito heads (Johnson et al. 2002). In a separate experiment, *Ae. aegypti* was intra-thoracically inoculated with CYD2 to overcome infection barriers in the midgut associated with oral feeding. The midgut tissue was poorly infected and although the viruses replicated, they did not disseminate to mosquito heads, implying that they are absent from the salivary gland and could not be transmitted during feeding (Johnson et al. 2002; Johnson et al. 2004).

6.3.4 Viremia in vaccines

112. In vaccinated humans, viremia is uncommon and rarely exceeds the level of PCR detection. CYD1 and CYD2 were not detected and only low titres of CYD3 and CYD4 were observed in humans (Morrison et al. 2010; Poo et al. 2011).

6.3.5 Shedding

113. The GM vaccine viruses were not shed in bodily fluids when tested in monkeys during pre-clinical studies (unpublished results, Sanofi).

114. Viral shedding in vaccinated humans is low and transient. During a phase III study, urine and saliva samples were tested for vaccine virus using RT-PCR. Vaccine virus was detected in only 2 of the 95 urine samples at levels close to the lower limit of quantification (unpublished results, conducted under licence DNIR-386). No replication-competent viruses were identified in these samples.

6.3.6 Stability in the environment and decontamination

115. Flaviviruses are labile in the environment due to their membrane envelopes, which are denatured by detergents and organic solvents. Methods of decontamination effective against the parent viruses are expected to be equally effective against the GMOs (see Section 5.2.5). In an unpublished study by Sanofi, the GM vaccine viruses were also found to be effectively inactivated after exposure to:

- 0.05 N sodium hydroxide for 45 minutes;
- Formaldehyde fumigation overnight for liquid virus samples;
- Dichloroisocyanurate (solution of 6340 ppm Novelty Chlor) for 2 minutes for dried virus samples.

116. Effective methods of physical decontamination are UV- and γ-irradiation (Burke & Monath 2001). The sensitivity to desiccation varies with each GM vaccine virus and desiccation alone is not considered an efficient decontamination method.

Section 7 Receiving environment

117. The receiving environment forms part of the context for assessing risks associated with dealings with GMOs (OGTR 2013). It informs the consideration of potential exposure pathways, including the likelihood of the GMOs spreading or persisting outside the site of release.

7.1 Site of release

118. The primary receiving environment would be the clinical facility where the vaccine is administered. Most clinical facilities would be equipped to deal with scheduled drugs and infectious agents and they typically comply with *AS/NZS 2243.3:2010 Safety in laboratories – Microbiological Safety and Containment* (Standards Australia/New Zealand 2010).

119. During vaccination of the patients, an aerosol of the GMO could be released into the clinical facility but the amount would be very small.

7.2 Related viral species in the receiving environment

120. The presence of related viral species may offer an opportunity for introduced genetic material to transfer horizontally from the GM vaccine strains to other organisms in receiving environment.

121. The more common mosquito-borne viruses in the Australian environment such as the Ross River virus, Barmah Forest virus and Chikungunya virus, are alphaviruses that are unrelated to the parent organisms.

122. The majority of mosquito-vectored flaviviruses in the Australian environment are sufficiently distantly related to the parent organisms that they are not transmitted by *Aedes* mosquitoes but by *Culex* mosquitoes. These include Murray Valley encephalitis virus, the Kunjin strain of West Nile virus and Japanese encephalitis virus.

123. *Aedes*-vectored flaviviruses that cause diseases in humans are rare in the Australian environment. The Edge Hill virus, which has 70% homology to DENV-2 (Blok et al. 1984), is the only member of the YFV sub-group that has been detected in Australia (Macdonald et al. 2010).

124. Outside Australia, YFV is most closely related to Sepik and Wesselsbron viruses while DENV is most closely related to Spondweni, Kedougou and Zika viruses.

7.3 Similar genetic material in the environment

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

126. All of the genes in the GM vaccine strains would be functionally similar to ones present in naturally occurring DENV. The genes introduced into the GM vaccine strains were derived from naturally-occurring isolates representing the four serotypes, and so similar genetic material would already be present in the environment.

7.4 Alternate hosts

127. Viruses are obligate parasites, which cannot replicate outside a host as they depend on the host's proteins for many replicative processes.

128. Australia does not have primates outside captivity that would support the sylvatic cycle of either YFV or DENV.

Section 8 Previous authorisations

8.1 Australian authorisations

129. No dengue vaccines have been approved for commercial use in Australia.

130. The Regulator issued a DNIR licence (DNIR-386) to conduct Phase IIa and Phase III clinical trials of Dengvaxia (then called ChimeriVax™-DEN) in Australia in March 2006. This licence expired on 31 December 2012.

131. The GMOs in Dengvaxia have the same YF 17D backbone as the Japanese encephalitis vaccine, IMOJEV. In 2010, IMOJEV was approved for use in Australia by the TGA and for commercial release by the Gene Technology Regulator (licence DIR 098).

8.2 International authorisations and experience

132. Dengvaxia was first commercially released in Mexico in December 2015. As of February 2017, it is available the countries listed in Table 2. To date, there are no reports of adverse events resulting from the transport, storage or disposal of Dengvaxia.

Table 2: Overseas marketing approvals for Sanofi's Dengvaxia

Approved in	Country
December 2015	Mexico, Philippines, Brazil
February 2016	El Salvador
June 2016	Costa Rica
August 2016	Guatemala, Paraguay, Indonesia
September 2016	Peru, Bolivia, Cambodia, Thailand
October 2016	Singapore
December 2016	Venezuela

Chapter 2 Risk Assessment

Section 1 Introduction

133. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs (Figure 6). Risks are identified within the established risk context (see Chapter 1) and take into account current scientific and technical knowledge. Uncertainty and in particular, knowledge gaps, is considered throughout the risk assessment process.

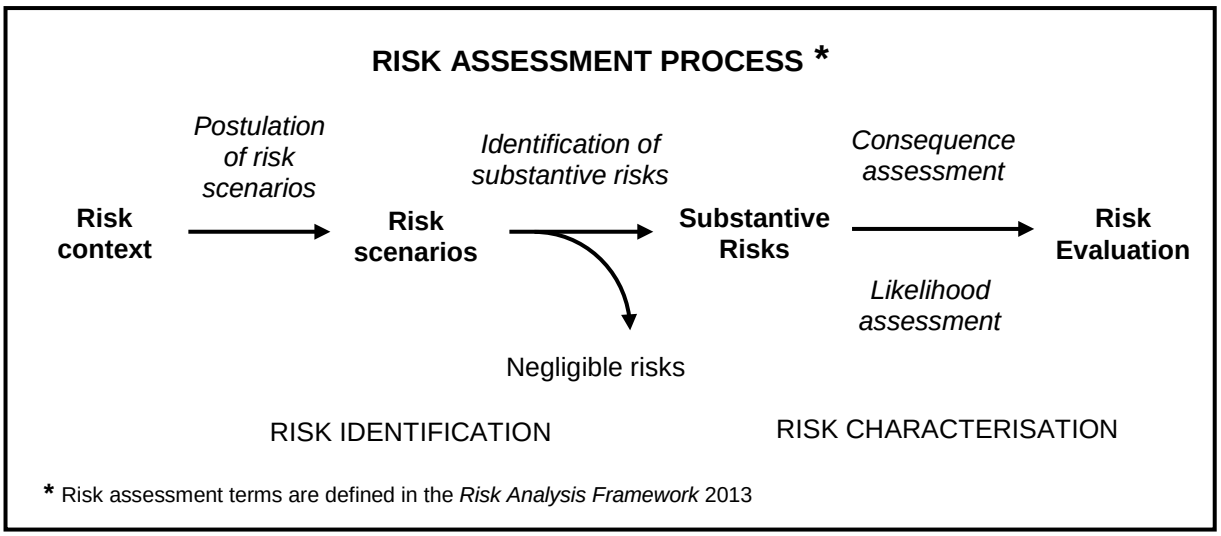

Figure 6. The risk assessment process

134. Risk identification first 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 whereby dealings with a GMO (risk scenarios) may, in the short and long term, harm people or the environment.

135. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. Substantive risks are further assessed 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.

136. Risk identification techniques used by the Regulator and evaluators at the OGTR include checklists, brainstorming, reported international experience and consultation (OGTR 2013). In conjunction with these techniques, risk scenarios postulated in RARMPs prepared previously for licence applications of the same and similar GMOs are also considered.

137. Substantive risks (i.e. those identified for further assessment) 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. Risk evaluation then combines the Consequence and Likelihood assessments to determine level of risk and whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk Identification

138. Postulated risk scenarios are comprised of three components (Figure 7):

i. Source of potential harm (risk source)

ii. Plausible causal linkage to potential harm (causal pathway) and

iii. Potential harm to an object of value (people or the environment).

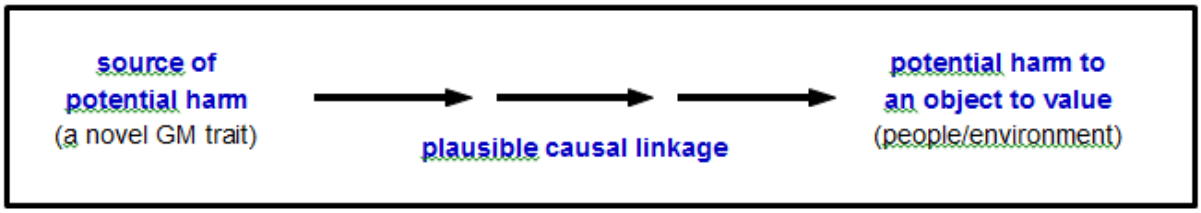

Figure 7. Components of a risk scenario

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

- the proposed dealings, which are import, transport or disposal of Dengvaxia and possession (including storage) in the course of any of these dealings
- restrictions placed on the import, transport or disposal of Dengvaxia by other regulatory agencies, the States and Territories
- characteristics of the parent organisms
- routes of exposure to the GMOs, the introduced genes and gene products
- potential exposure to the same genes and gene products from environmental sources
- the release environment and
- practices during and after administration of Dengvaxia.

140. The TGA would regulate quality, safety and efficacy of Dengvaxia under the *Therapeutic Goods Act 1989*, as mentioned in Chapter 1 Section 2.1. This would include:

- assessment of patient safety, vaccine quality and efficacy prior to inclusion on the ARTG
- recommended practices for the transport, storage and disposal of the GM vaccine strains under the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8*
- requirements for the scheduling, labelling and packaging under the *Poisons Standard.*

141. Use of GMOs for therapeutic purposes is not regulated under the Act, and the Regulator does not assess risks from their use, such as risks associated with needlestick injury of medical staff during vaccination or risks to other people from contact with the vaccinee.

142. The current assessment focuses on risks posed to people or the environment, including long term persistence of the GMOs, which might arise from the import, transport, storage or disposal of Dengvaxia.

143. The GMOs could persist in the environment through sustained transmission by infected vectors to susceptible hosts, and this could increase the likelihood of exposure. However, dissemination of the vaccine strains by mosquitoes is not probable as viremia in vaccinees is both transient and low (Higgs et al. 2006). Furthermore, when fed viremic blood during an experiment, the GMOs did not disseminate to the mosquito's salivary glands, and are unlikely be transmitted during feeding (Johnson et al. 2002; Guy et al. 2010a). Therefore, persistence through sustained transmission is not considered further.

144. Recombination of the GMOs with a circulating virus to generate a novel virus that would survive in the environment is not probable. Recombination of DENV, which are single stranded RNA viruses, is very rare and requires a mosquito be co-infected with more than one strain of DENV. This is not probable given DENV is not endemic in Australia. Recombination of the GMOs with a different flavivirus is not likely since recombination of different flaviviruses has not been recorded and was not observed during co-infection (Dupont-Rouzeyrol et al. 2015). Flaviviruses do not circulate widely in

Australia and the most common flaviviruses in Australia are vectored by the *Culex* mosquito while DENV is vectored by the *Aedes* mosquito. Furthermore, the genes and regulatory sequences in Dengvaxia are derived from a YFV strain that has been widely used for over 60 years or from naturally occurring DENV strains (see Chapter 1 Section 5.6.1). Therefore, recombination is not considered further.

2.1 Postulated risk scenarios

145. Two risk scenarios were postulated, as summarised in Table 3. These risk scenarios were evaluated considering both short and long term effects, restrictions imposed by DAWR and the TGA, and in the context of practices proposed by the applicant. Detailed evaluations of these scenarios are provided later in this section. None of the risk scenarios were identified as a risk that could be greater than negligible and warranting further scrutiny.

Table 3. Summary of risk scenarios from dealings with GM Dengvaxia

#	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	GM vaccine strains in Dengvaxia	Unintentional release of GMOs during transport or storage ⬇ Person is exposed to GMOs ⬇ Person is infected with GMOs	Ill health	No	• Dengvaxia would be transported under guidelines that are mandated by the States and Territories. • Storage will be at secure storage or clinical facilities. • The GMOs are attenuated in their ability to replicate efficiently. • Exposure leading to infection requires entry of GMOs by injection or through broken skin. • The dose received through accidental exposure would be far smaller than that administered during vaccination.
2	GM vaccine strains in Dengvaxia	Used or unused vials of Dengvaxia and syringes contaminated with Dengvaxia disposed in medical waste ⬇ Person exposed to the GMOs via sharps injury during waste disposal ⬇ Person is infected with GMOs	Ill health	No	• Used, unused or expired Dengvaxia would be disposed as medical waste by healthcare workers and distributors. • Inadvertent exposure during disposal would be minimised by well-established procedures for disposal. • The GMOs are highly labile in the environment, and would not survive for long in waste. • Exposure leading to infection requires entry of GMOs by injection or through broken skin. • The dose received through accidental exposure would be far smaller than that administered during vaccination.

Risk scenario 1

Risk source	GM vaccine strains in Dengvaxia
Causal pathway	⬇ Unintentional release of GMOs during transport or storage ⬇ Person is exposed to GMOs ⬇ Person is infected with GMOs ⬇
Potential harm	Ill health

146. The GMOs are susceptible to common chemical decontaminants such as detergents and hypochlorite.

147. Dengvaxia must be stored between 2-8°C and requires a cold chain, which is a well-controlled and uninterrupted sequence of transport and storage to maintain the vaccine in this narrow temperature range, to preserve the potency of the vaccine.

148. Sanofi proposes to import the vaccine as a freeze-dried powder in a sealed glass vial, and this combination is not likely to leak. These packs would be packaged in secondary cartons and the cartons packed in corrugated cardboard shipping cartons for distribution (Chapter 1 Section 4). Transport of Dengvaxia between the port of entry and the warehouse would continue in this packaging.

149. Vaccines are classified as Schedule 4 medicines. The *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) recommends that:

- upon arrival, packaging should be removed, and stock should be examined for the absence of damage or evidence of tampering. Damaged stock should be quarantined.
- packaging and handling of cold chain medicines should alert the receiver of its contents and that the receiver should place the medicines in appropriate storage facilities as soon as possible, and
- wholesalers ensure that persons supplied with medicines are authorised appropriately under State or Territory legislation to be supplied with those medicines.

150. Additionally, storage, handling and transport would be in accordance with both the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) and the WHO's Good Distribution Practices for pharmaceutical products (WHO 2010). These guidelines require that:

- written procedures for dealing with spillage of items of special hazard are available and training provided to responsible staff
- in the event of a spill, the spill should be cleaned up promptly and rendered safe as quickly as practicable in accordance with the material safety data sheet (MSDS)
- spills kits should be conveniently located within the storage area
- access is restricted to individuals with the appropriate training .

These practices would lower the probability of unintended dispersal of the GMOs.

151. These practices would minimise the chances of damaged and leaking stock going unnoticed and increase the chances of Dengvaxia being handled by individuals who would know how to decontaminate a spill, thus minimising the probability of unintended dispersal of the GMOs.

152. Should the GMOs be unintentionally released, they are highly unlikely to infect people or animals as they cannot replicate outside a host, are fragile in the environment and are readily decontaminated. In addition, the GMOs are restricted in replication and would be less pathogenic than

circulating DENV. The Product Information on Dengvaxia advises that the most common side effects from after vaccination are headache, myalgia, malaise, fever and general loss of energy. Like infection with other flaviviruses, vaccination with the GMOs does not result in latent infection or integration into the host genome as there is no DNA intermediate.

153. Exposure leading to infection requires entry of GMOs by injection or through broken skin. Injection of the GMO would not eventuate from transport. Should a vial break, it is not probable that sufficient GMO would enter through broken skin to result in an adverse reaction.

154. The dose received through accidental exposure would be far smaller than that administered during vaccination. A single dose of Dengvaxia, administered by injection, results in low and transient viremia. The small amount received through broken skin would be far less than a single dose and unlikely to lead to ill health.

155. Therefore, even if an individual or animal is inadvertently exposed to the GMOs, they are unlikely to cause disease.

156. ***Conclusion***: Risk scenario 1 is not identified as a substantive risk. Import, transport and storage procedures will minimise the likelihood of unintended dispersal of the GMOs, it is highly unlikely that inadvertent release of the GMOs would result in persons or animals being exposed in a manner that would result in ill health, and the GMOs are attenuated. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Risk scenario 2

Risk source	GM vaccine strains in Dengvaxia
Causal pathway	⬇ Used or unused vials of Dengvaxia and syringes contaminated with Dengvaxia disposed in medical waste ⬇ Person exposed to the GMOs via sharps injury during waste disposal ⬇ Person infected with GMOs ⬇
Potential harm	Ill health

157. The GMOs are highly susceptible to desiccation due to their lipid envelope and would not remain viable for extended periods on open surfaces.

158. The cold chain, which is intended to preserve the potency of the vaccine, requires cold packaging/refrigeration and this adds a level of containment during import, storage and transport.

159. Individuals may be inadvertently exposed to GMOs while disposing of used, expired, or unused vials of Dengvaxia. The two locations where this is most likely to occur are at:

- distribution warehouses where stocks of Dengvaxia are held
- locations where Dengvaxia is administered.

160. Healthcare professionals routinely dispose of medical waste and would have standardised procedures for the safe disposal of both used vials with residual Dengvaxia and expired vials of Dengvaxia. This is required by the State governments, which have issued operational guidance documents for the disposal of biohazardous waste to reduce potential risk to waste handlers.

161. Dengvaxia is supplied in a vial made from a type of glass that is commonly used for packaging parenteral drug products as it does not shatter easily.

162. At the distribution facility, there may be vials of Dengvaxia which are past their expiry date but which may still contain viable GMOs. These sealed vials would be decontaminated by a waste contractor and all stock destroyed would be recorded. Given the GMOs would still be a dry powder in a sealed vial during waste disposal, the waste handlers are highly unlikely to be exposed to Dengvaxia in a manner that would lead to productive infection.

163. The *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) requires:

- specific training for personnel handling medicines that pose high risk to personnel if package integrity is breached or spillage occurs
- waste medicines be collected and destroyed by a person who is licensed or permitted to do so under relevant State or Territory legislation
- medicines for destruction be enclosed in sealed packaging or in a container.

164. Dengvaxia must be stored between 2-8°C to maintain vaccine potency. Dengvaxia slated for destruction would be held at ambient temperature. Unpublished studies by the applicant found that the cell culture infectious dose fell by 10^7 over a 7-day period at room temperature.

165. Exposure leading to infection requires entry of GMOs by injection or through broken skin. Infection control policy in States/Territories requires cuts and abrasions on exposed skin be covered with water-resistant occlusive dressing. For productive infection, individuals must be exposed to an infectious dose. Residual liquid in used vials would not contain a sufficient titre to cause productive infection. The same would apply to secondary waste such as gloves that may be contaminated with the vaccine. The GMOs cannot replicate outside a host cell, so viruses in the used vials could not multiply to reach an infective dose.

166. **Conclusion**: Risk scenario 2 is not identified as a substantive risk. Given the methods of disposal for material that may be contaminated with the GMOs proposed for the various locations, exposure to the GMO leading to ill health is unlikely. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

167. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis [2].

168. There are several types of uncertainty in risk analysis (Bammer 2008; Clark 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity

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

- perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

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

170. Uncertainty can also arise from a lack of experience with the GMO itself. However, with Dengvaxia the overall level of uncertainty is low given the clinical trials conducted in many countries including Australia, and the commercial availability of Dengvaxia in many countries. There have been no reports of SAEs resulting from the usage of Dengvaxia.

171. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

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

Section 4 Risk evaluation

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

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

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

175. Two risk scenarios were identified whereby the proposed dealings might give rise to harm to people of the environment. This included consideration of how people may be exposed to the GM viral strains in Dengvaxia, potential health effects in people who are inadvertently exposed, potential for persistence of the GMOs through sustained transmission and the potential for viral recombination. The opportunity for gene transfer and its effects, if this occurred, were also considered.

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

177. In the context of the control measures proposed by the applicant and the operating guidelines of the pertinent regulatory agencies, and considering both the short and long term, neither of these scenarios was identified as substantive risks requiring further assessment. The principal reasons for this include:

- exposure to the proposed Dengvaxia would be minimised by well-established clinical, import, transport, storage and disposal procedures
- survival outside of a host is limited to short periods, and it is susceptible to common chemical decontaminants
- the GM vaccine strains are attenuated
- the GM vaccine strains are not transmitted by mosquitoes.

178. Therefore, any risks posed to the health and safety of people, or the environment, from the proposed release of Dengvaxia are considered to be negligible. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

179. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring mitigation; it considers limits and controls proposed by the applicant together with general risk management measures. The risk management plan informs the Regulator's decision-making process and is affected through licence conditions.

180. 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.

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

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

Section 2 Risk treatment measures for substantive risks

183. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed dealings with Dengvaxia. These risk scenarios were considered in the context of the proposed receiving environment and the Australia-wide release. The risk evaluation concluded that no controls are required to treat the negligible risks.

Section 3 General risk management

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

- applicant suitability
- identification of the persons or classes of persons covered by the licence
- reporting structures
- a requirement that the applicant allows access to specified sites for purpose of monitoring or auditing.

3.1 Applicant suitability

185. 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 capacity of the applicant to meet the conditions of the licence.

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

187. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

3.2 Testing methodology

189. Sanofi must provide the Regulator with a method to reliably detect the presence of GM vaccine strains and introduced genetic material in humans. This instrument is required prior to conducting any dealings authorised by the licence.

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

190. Any person, including the licence holder, may conduct any dealing with the GM vaccine permitted by the licence.

3.4 Reporting requirements

191. 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 dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

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

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

3.5 Monitoring for Compliance

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

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

Section 4 Post release review

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

197. For the current DIR licence application, 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 would 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).

198. 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

199. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO 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 a review of a RARMP (see Section 4.3 below) as well as the RARMPs of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

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

201. 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.

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

203. Characterisation of the risk scenarios discussed in Chapter 2 did not identify any risk levels that were greater than negligible. The risks were not considered substantive and warranting further detailed assessment. The uncertainty associated with the proposed dealings is considered low and no specific indicators of harm have been identified in this RARMP for application DIR 148. However, specific indicators of harm may be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

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

4.3 Review of the RARMP

205. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial

risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

206. The risk assessment concludes that this proposed commercial release of Dengvaxia pose negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

207. However, general licence conditions have been imposed to ensure ongoing oversight of the commercial release.

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Appendix A Summary of submissions on RARMP preparation from experts, agencies and authorities

Before commencing preparation of the RARMP, the Regulator requested 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 how they are addressed in the consultation RARMP, are summarised below.

Sub #	Summary of issues raised	Comment
1	The Regulator should consider the potential for spread of the vaccine virus via blood/organ donation pathways, and the need to monitor seroconversion should inadvertent exposure occur.	Risks associates with direct use of the vaccine would be considered by the TGA. Inadvertent exposure as a result of a spill during transport or storage, or during waste disposal was assessed to be a negligible risk.
2	**Environmental fate**: GM virus may enter the environment by accidental release during transport or incorrect disposal. The vector for disease transmission is endemic in areas of Australia. Risk is likely to be low as the applicant indicates GM viruses have limited survival outside host or vector. However data on environmental fate, survival and persistence of the GM viruses should be included in the RARMP.	Environmental stability is addressed in Chapter 1 Section 6.3.6. Risks associated with accidental release are considered in Chapter 2.
	Genetic stability: the risk of unintended changes to GM viral characteristics may depend on genetic stability. Genetic stability of attenuating mutations should be assessed in the RARMP, together with the risk of reversion to wild-type strains. Notes that there is little risk as the genetic stability appears to be confirmed.	The genetic modification involves the replacement of two genes rather than point mutations. Reversion would require acquiring yellow fever virus genes from the Australian environment which is improbable.
	Recombination: theoretically, recombination can occur with wild-type flaviviruses in the vector or host. If intended for travellers, interaction with other vaccine viruses may need to be considered. Other viruses carried by mosquitos include Zika, Chikungunya and West Nile virus. The risk is considered low as recombination between flaviviruses is contested and laboratory recombinants are attenuated. However the WHO's guidelines for Dengue vaccines recommend assessing recombination.	Interaction between vaccine strains arising from the use of the vaccine falls within the regulatory responsibility of the TGA. Recombination between flaviviruses arising from transport storage or disposal is considered in Chapter 2 and was not considered to present a plausible risk.
	Host range: Dengue and yellow fever viruses replicate in an arthropod vector and vertebrate host. Potential risk to non-target organisms in the environment is considered negligible. However, genetic changes in viruses can result in changes in host range. Changes to host range or vector species may need to be considered.	The genetic modification results in chimeric viruses. It does not alter individual genes and in particular, it does not alter the envelope protein (E) which is the primary determinant of host range. Furthermore, given that both parent organisms have the same host range, the host range would be expected to remain the same in the GM vaccine strains. While many non-primate species can be infected, they are not classified as hosts as the maximum level of viremia is below

Sub #	Summary of issues raised	Comment
		transmission thresholds.
	Recommendations: Notes that the risks to the environment are likely to be negligible due to limited survival outside the host or vector, a single host and the history of safe use of the parent yellow fever virus in vaccines. However any risks of changed viral characteristics that could result in changes to survival, virulence or host range should be assessed in the RARMP.	Given the considerations above, risks to the environment from the GM vaccine strains are likely to be negligible.
3	No objections to the research embodied in the application	-
4	No comment	-
5	Council does not have a specialist scientific expert to make an assessment and will not provide comments.	-
6	Council does not generally support genetically modified products due to environmental and economic risks (some as yet unknown) that could negatively impact Australian communities. They support Australia being "green and pure" for health and marketing advantages. However, in this instance, there may be some significant public health benefits and the vaccine is not formally opposed. Request lack of formal support and accompanying comments be considered and noted.	Marketing and trade issues, as well as potential benefits, are outside the scope of assessments conducted by the Regulator.

Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP

The Regulator received a number of submissions from prescribed experts, agencies and authorities[3] on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Sub #	Summary of issues raised	Comment
1	Consider advising TGA about risks to people that may be accidentally exposed to the vaccine.	The consultation RARMP has been made available to the TGA. The TGA would also conduct its own safety assessment for Dengvaxia and its registration on the ARTG. There have been no overseas reports of adverse events resulting from the import, transport, storage and disposal of Dengvaxia.
2	Consider adverse events or incidents reported overseas.	—
3	Does not have a dengue fever program.	—
4	No concerns. Agrees with negligible risk to people and the environment.	—
5	Supports conclusion that the dealings pose negligible risk of harm to human health and safety and to the environment.	—
6	No objections to the application. Notes licence conditions to ensure ongoing oversight of the release and that the release is subject to TGA approval.	—

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

Appendix C Summary of submissions from the public on the consultation RARMP

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

Sub #	Summary of issues raised	Comment
1	Vaccination with Dengvaxia is not advised by the WHO if less than half the population has had the disease, as is the case in Australia. It increases the risk of hospitalisation in vaccinated children aged 2-5 years. Dengvaxia should not be released in Australia until it is made safe.	Dengvaxia is intended for individuals travelling to dengue-affected areas. Dengvaxia is not recommended for children under the age of 9. Safety and efficacy of the vaccine falls within the regulatory responsibility of the TGA.
2	Wants to know the side effects of Dengvaxia, the possibility of mosquito transmission, and the spreading dengue through shedding.	The TGA would assess the safety of Dengvaxia, including side effects and adverse reactions, prior to its inclusion on the ARTG. The potential for transmission through mosquitoes or shedding is addressed in Chapter 1, Section 6.3.
3	Wants to know whether the Dengue virus is injected, the adverse reactions to Dengvaxia, and whether viral shedding is an issue.	Delivery of the vaccine is addressed in Chapter 1, Section 3. The TGA would assess the safety of Dengvaxia, including side effects and adverse reactions, prior to its inclusion on the ARTG. Shedding and transmissibility are addressed in Chapter 1, Section 6.3.
4	Wants to know whether the vaccine is injected, what the adverse reactions are, and whether shedding of the virus will spread it throughout Australia.	Delivery of the vaccine is addressed in Chapter 1, Section 3. The TGA would assess the safety of Dengvaxia, including side effects and adverse reactions, prior to its inclusion on the ARTG. Shedding and transmissibility are addressed in Chapter 1, Section 6.3.
5	Has huge safety concerns about the wide ranging adverse health implications of the vaccine on the Australian public. Adverse reactions from individual vaccine components, combined vaccine components, shedding properties, and the effect of Dengvaxia on other vaccines should be independently assessed.	The TGA would assess the safety of Dengvaxia, including side effects, adverse reactions and excipients, prior to its inclusion on the ARTG. Shedding and transmissibility are addressed in Chapter 1, Section 6.3.
6	Considers all vaccines pose some risk, either short or long term. Wants to know whether Dengvaxia contains Dengue virus, the adverse reactions associated with Dengvaxia, and whether shedding is an issue causing the virus to spread amongst the Australian people resulting a pandemic of dengue fever.	The GMOs in Dengvaxia are detailed in Chapter 1, Section 6 of the RARMP. The TGA would assess the safety of Dengvaxia, including side effects and adverse reactions, prior to its inclusion on the ARTG. Shedding and transmissibility are addressed in Chapter 1, Section 6.3.

Sub #	Summary of issues raised	Comment
7	Would welcome the registration and licensing of a safe and effective vaccine against dengue. Notes the current availability of Dengvaxia in 14 other countries, its potential to reduce incidence of severe re-infection, and the low risk finding of the Regulator's risk assessment. Use of Dengvaxia should be limited to individuals travelling to countries where dengue is endemic, as per WHO recommendations. Clarify use of Dengvaxia by pregnant women and the immunocompromised.	Recommendations on the conditions for use of Dengvaxia are determined by the TGA, prior to inclusion of the vaccine on the ARTG.