



Australian Government

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

# Risk Assessment and Risk Management Plan (Consultation version) for

## DIR 207

### Commercial release of a genetically modified (GM) mosquito strain to help prevent dengue outbreaks

Applicant: Oxitec Australia Pty Ltd

14 May 2025

#### **This RARMP is open for consultation until 7 July 2025**

Written comments on the risks to human health and safety and the environment posed by this proposed supply of the GM mosquito strain to help prevent dengue outbreaks are invited. You may make your submission via:

consultation hub: <https://consultations.health.gov.au/ogtr/dir-207-consultation>

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

email to: [ogtr@health.gov.au](mailto:ogtr@health.gov.au).

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

# Summary of the Risk Assessment and Risk Management Plan (Consultation Version) for Licence Application DIR 207

## Introduction

The Gene Technology Regulator (the Regulator) has received a licence application (DIR 207) for the commercial supply of a strain of genetically modified (GM) mosquitoes to help control the population of *Aedes aegypti* mosquitoes in Queensland. These activities are classified as Dealings involving the Intentional Release (DIR) of a genetically modified organism into the Australian environment under the *Gene Technology Act 2000*.

Before the commercial release of the GM *Ae. aegypti*, Oxitec Australia Pty Ltd must also obtain regulatory approval from the Australian Pesticides and Veterinary Medicines Authority (APVMA). The APVMA administers the *Agricultural and Veterinary Chemicals Code Act 1994* (the Agvet Code) to regulate agricultural and veterinary chemical products, including biological pest control agents. Approvals would also be required from other agencies: the GM mosquito would need approvals prior to importation from both the Department of Agriculture Fisheries and Forestry (DAFF) and the Department of Climate Change, Energy, the Environment and Water (DCCEEW). The Queensland Government would have a role in the authorisation of the release of these GM mosquitoes in Queensland.

For the ongoing commercial supply of the GM mosquito, the dealings assessed by the Regulator are to:

- (a) import the GM mosquito;
- (b) transport the GM mosquito;
- (c) conduct experiment with the GM mosquito (collect and analyse samples and perform demonstrations of the technology);
- (d) grow, raise or culture the GM mosquito;
- (e) dispose of the GM mosquito;

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

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

## The application

<b>Application number</b>	DIR-207
<b>Applicant</b>	Oxitec Australia Pty Ltd
<b>Project title</b>	Commercial release of a genetically modified (GM) mosquito strain to help prevent dengue outbreaks <sup>1</sup> .

<sup>1</sup> Application title as provided by the applicant: *Aedes aegypti* mosquito strain OX5034 for commercial release in the state of Queensland

<b>Parent organism</b>	Mosquito ( <i>Ae. aegypti</i> )
<b>Genetic modifications</b>	<ul style="list-style-type: none"> <li>• Expression of a self-limiting gene to prevent female mosquito larvae from surviving to adulthood.</li> <li>• Expression of a red fluorescent marker gene allowing for easy identification of the GM mosquitoes in the laboratory.</li> </ul>
<b>Previous releases</b>	Field trials have been conducted in Brazil and the United States of America (USA).
<b>Current approvals</b>	The product is approved and commercially available in Brazil.
<b>Proposed locations</b>	Queensland
<b>Primary purpose</b>	Commercial release of GM mosquitoes for the purpose of decreasing the population of <i>Ae. aegypti</i> mosquitoes to control dengue in Queensland.

## Risk assessment

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

Credible pathways to potential harm that were considered from the proposed release of the GM *Ae. aegypti* include; the potential exposure of people and animals to the GMO and the potential effect of the GMO as a result of its release into the environment.

The risk assessment concludes that risks to both the health and safety of people and the environment from the proposed supply of GMO are negligible. No risk treatment measures are included in the licence to maintain the risk context.

The principal reasons for the conclusion of negligible risks associated with the proposed dealings conducted with the GMO are:

- The GM mosquitoes have a limited lifespan (around 7 days). Without continuous release of the GM mosquitoes, their numbers will decrease rapidly, and they will not persist in the environment;
- Male mosquitoes are unable to transmit disease as they do not bite humans or animals;
- There are no species of animals in Australia that rely solely on mosquitoes for survival;
- Mosquitoes are not an essential pollinator of plants;
- The proteins encoded by the transgenes in the GM mosquitoes are not toxic or allergenic; and
- The GM mosquitoes are still susceptible to standard insecticides.

The GM mosquitoes would require approval for importation under the *Biosecurity Act 2015*. An import permit imposes specific import conditions to manage biosecurity risks. This includes the assessment of the impact of the introduction of the organism into Australia.

The GM mosquito will also be assessed for its suitability for import under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) to include the species on the *List of specimens taken to be suitable for live import* (the Live Import List) by the Department of Climate Change, Energy, the Environment and Water.

The GM mosquito would need to be registered or granted a permit by the APVMA, who would impose conditions on supply, use, transport, storage and disposal of the GM mosquitoes under the *Agricultural and Veterinary Chemicals Code Act 1994* (AgVet Code). The quality, safety and efficacy of the biological pest control agent will be assessed, including environmental toxicological and pathogenicity/infectivity studies to non-target organisms, as part of the environment risk assessment.

### ***Risk management***

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

The risk management plan concludes the negligible risks can be managed to protect the health and safety of people and the environment.

General and additional reporting conditions were included in the draft licence to ensure that there is ongoing oversight of the GM mosquitoes. Conditions were also included requiring the applicant to report any new information obtained after release of the GM mosquitoes to allow the collection of information to verify the findings of the RARMP. The draft licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and other reporting requirements, which include an obligation to report any unintended effects.

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## Abbreviations

AcNPV	<i>Autographa californica</i> nuclear polyhedrosis virus
ACT	Australian Capital Territory
AgVet Code	<i>Agricultural and Veterinary Chemicals Code Act 1994</i>
ALE	Average life expectancy
<i>ampR</i>	Ampicillin resistance
APVMA	Australian Pesticides and Veterinary Medicines Authority
DAFF	Department of Agriculture, Fisheries and Forestry
DCCEEW	Department of Climate Change, Energy, the Environment and Water
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
EPA	Environment Protection Authority
EPBC	Environment Protection and Biodiversity Conservation
g	gram
GFP	Green fluorescent protein
GLP	Good Laboratory Practice
GM	Genetically modified
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
HGT	Horizontal gene transfer
kg	kilogram
LGA	Local government area
LOD	Limit of detection
mg	milligram
ml	Milli litre
ND	Not detectable
ng	nanogram
NLS	Nuclear localisation sequence
NNDSS	National Notifiable Disease Surveillance System
NSW	New South Wales
NT	Northern Territory
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase chain reaction
PubCRIS	Public Chemical Registration Information System
pg	picogram
QLD	Queensland
RAF	Risk analysis framework

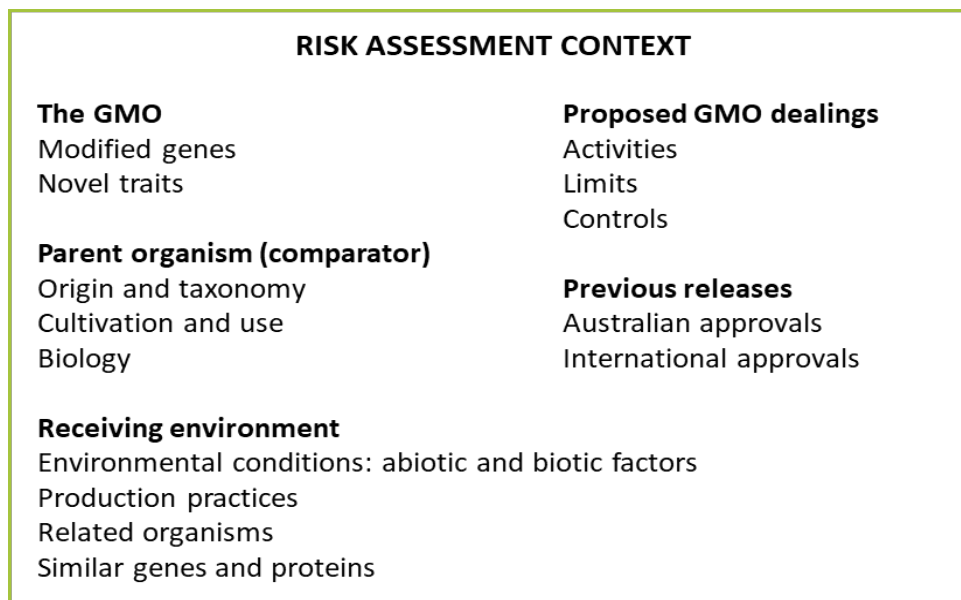
RARMP	Risk Assessment and Risk Management Plan
rDNA	Recombinant DNA
RNA	Ribonucleic acid
SA	South Australia
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIT	Sterile insect technique
TAS	Tasmania
tetO	Tetracycline operators
tetR	Tetracycline repressor
the Act	The <i>Gene Technology Act 2000</i>
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
tTA	Transactivator protein
tTAV	Transactivator protein variant
UK	United Kingdom
US EPA	United States Environmental Protection Agency
USA	United States of America
VIC	Victoria
WA	Western Australia
WHO	World Health Organisation
WT	Wild type
WWTP	Waste water treatment plant



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



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

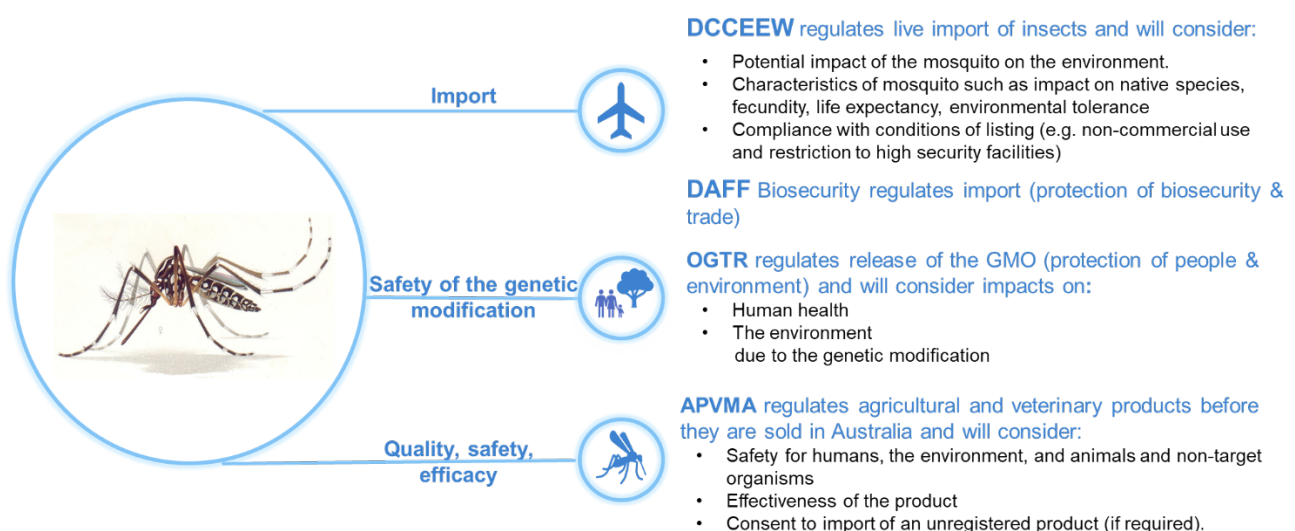
6. Since this application is for commercial purposes, it does not meet the criteria for a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on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

Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities and agencies prescribed in the Regulations and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

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

### 1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. For this application, the role and responsibilities of the OGTR and other agencies responsible for issuing authorisation for this product are summarised in Figure 2. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Department of Agriculture, Fisheries and Forestry (DAFF) and Department of Climate Change, Energy, the Environment and Water (DCCEEW).



**Figure 2 Roles and responsibilities of OGTR and other regulatory agencies involved with the GM mosquito.**

9. The proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

10. For the commercial supply of GM mosquitoes as a biological pest control agent, dealings regulated under the Act include the import, transport, storage, conducting experiments (collection and analysis of field samples, and demonstration of the technology), the rearing of the GM mosquitoes and disposal of GM mosquitoes. The Regulator has assessed risks associated with the release of the GM mosquitoes including risks to human health and to the environment. This includes assessing the risk of persistence of the GM mosquitoes in the environment and the impact of the release on the transmission of arboviruses.

11. In accordance with the *Gene Technology Act 2000*, the OGTR assessment can only consider the risk associated with gene technology and the modification made to the parent organism, in this case *Ae. aegypti*. As a result, some aspects of this release were not considered but they would be part of the evaluation and approval process of other government agencies. Aspects not considered in this RARMP include:

- the efficacy of the proposed release to control transmission of dengue in Queensland,
- the risk/benefit analysis associated with this release,
- the qualitative comparison of methods to control transmission of dengue,

- the presence of *Ae. aegypti* in States and Territories, other than Queensland and
- the strain of *Ae. aegypti* that has been modified and proposed to be released (the strain has been discussed in the RARMP for the purpose of context).

12. The APVMA provides a national registration and permit scheme for agricultural and veterinary chemical products. It administers the provisions of the *Agricultural and Veterinary Chemicals Code Act 1994* (AgVet Code).

13. The APVMA regulates the quality, safety and efficacy, and trade risks associated with the GM mosquitoes under the AgVet Code. This includes safety and efficacy of the biological pest control agent; environmental risks; and recommended practices for the use, transport, storage and disposal of the GMO. The APVMA assesses all pesticide / pest control use in Australia and sets conditions of use.

14. Before a biological pest control agent is released into the Australian environment, a risk analysis must be undertaken by DAFF under the *Biosecurity Act 2015* to determine whether the risk of release is considered acceptable. This risk analysis would include host specificity and the impact of the introduction of a new strain of *Ae. aegypti* into Australia. Import permits may require specific tests to be conducted in approved arrangement premises prior to release to prevent potential impacts of the species in the Australian environment.

15. Approval would also be needed from DCCEEW under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) to include the species in Part 1 of the *List of specimens taken to be suitable for live import* (the Live Import List).

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

## **1.2 *Aedes aegypti* control and surveillance program**

### **1.2.1 Control measures for the prevention of mosquito-borne disease**

17. One of the main methods of controlling the spread of these diseases is by controlling the population of *Ae. aegypti* or its ability to carry the viruses that causes these diseases (vector control). Vector control can be carried out via environmental methods (e.g. cleaning the environment, waste water management schemes); chemical methods (e.g. use of larvicides/insecticides); biological methods (e.g. release of sterile males, biological agents that eat larvae and endosymbionts like *Wolbachia* that disrupt the transmission of arboviruses) (OECD, 2018; Beebe et al., 2021; Ogunlade et al., 2021; Sanchez-Aldana-Sanchez et al., 2023).

#### Environmental methods of mosquito control

18. Environmental methods of mosquito control involve the removal of potential mosquito breeding sites. This includes activities such as tipping out any water from plastic containers, buckets or tarpaulins; keeping bins covered; and throwing out any rubbish that could potentially retain water such as tyres, empty containers or pots.

19. Ultimately, one of the methods to control outbreaks of dengue is to educate the community to remove potential breeding sites on their properties and prevent themselves from being bitten by mosquitoes through personal protective measures. The Queensland government has recommendations and tips on removing potential mosquito breeding sites. There is also a provision in the Public Health Act 2005 (Queensland) that property owners are obliged to preclude mosquito breeding on their properties as mosquitoes are 'designated pests'.

#### Sterile insect techniques

20. The sterile insect technique (SIT) is the release of male mosquitoes that have been sterilised by either irradiation, chemicals or genetic modification (Flores and O'Neill, 2018; Rahul et al., 2024). These

sterile male mosquitoes would then breed with WT female mosquitoes but not produce any offspring thus reducing the numbers of target mosquitoes.

21. In Australia, SIT has been used to control Mediterranean fruit fly (*Ceratitidis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Department of Agriculture Fisheries and Forestry, 2023b). Based on publicly available data, there has not been any SIT involving sterile mosquitoes in Australia.

#### Chemical control of mosquitoes

22. The traditional method to control outbreaks of mosquito-borne disease, such as dengue, has been to target breeding sites and use insecticides in areas where population of vectors were more concentrated. The Queensland government provides advice for pest management technicians regarding the areas to target both inside and outside houses and the type of insecticide efficient against *Ae. aegypti* (Queensland Government, 2017). The insecticide recommended are synthetic pyrethroids mixed with water.

#### The use of *Wolbachia* to control mosquitoes

23. *Wolbachia* is an intracellular symbiotic bacterium which is maternally inherited. It is found in a wide range of insects and some *Wolbachia* have been shown to prevent transmission of viruses (Kaur et al., 2021). *Wolbachia* has a broad impact on the mosquito and its intracellular processes to favour its own transmission and survival. As a result of the changes to intracellular processes, it can inhibit viral replication, so the mosquito can no longer transmit viral diseases such as dengue or Zika. For this reason, *Wolbachia* strain *wMel* was used as a biological pest control strategy to manage dengue transmission in northern Queensland (O'Neill et al., 2018; Ryan et al., 2019). This method consists of the release of *Ae. aegypti* mosquitoes containing *wMel*, with the objective to transmit this *Wolbachia* strain to as many *Ae. aegypti* mosquitoes as possible in the wild. This strategy has been successful in the reduction of dengue transmission in the areas where mosquitoes containing *Wolbachia* were released (O'Neill et al., 2018; Ryan et al., 2019). As of 2023, there have been releases covering 300 km<sup>2</sup> of northern Queensland with a population of over 300,000 people (for further information see World Mosquito Program (Australia), accessed 24 January 2025).

24. The presence of *Wolbachia* in mosquitoes can also be used to reduce the population of *Ae. aegypti* through a mechanism called cytoplasmic incompatibility, where matings between mosquitoes with different *Wolbachia* infection status can result in death in the mosquito embryos (see paragraph 201 and Figure 14 for further details).

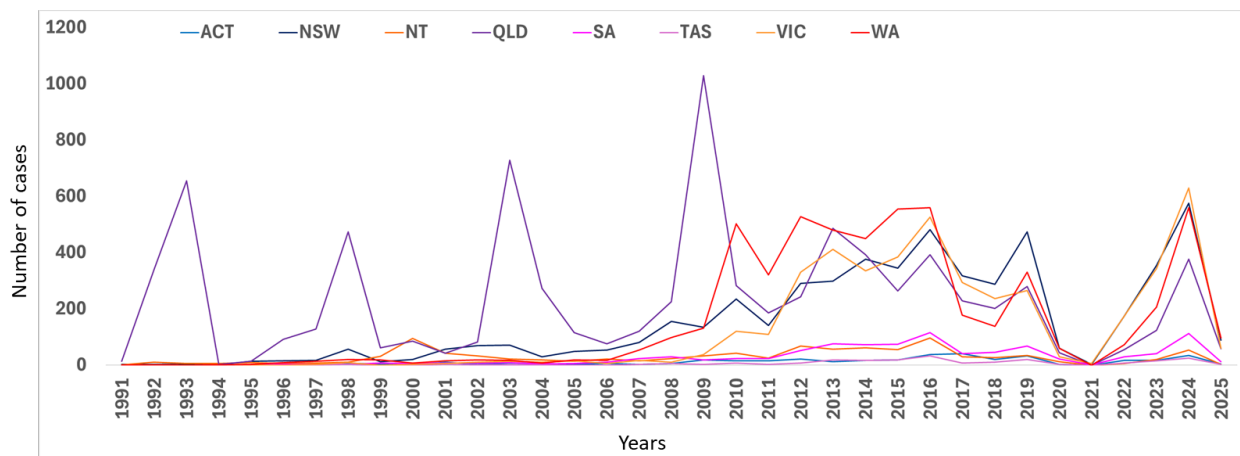
#### **1.2.2 Surveillance programs in Australia**

25. In Australia, the mosquito borne viruses dengue, Zika, chikungunya yellow fever and Ross-River viruses are listed as a notifiable disease under the National Health Security (National Notifiable Disease List) Instrument 2018, which is made under the National Health Security Act 2007. Dengue fever is the second most frequently reported disease in comparison to diseases cause by Zika, chikungunya, yellow fever and Ross River viruses based on data obtained between 1991 and 2025 (National Notifiable Disease Surveillance System (NNDSS)).

26. Between 1991 and 2009, there were spikes of dengue fever cases in Australia, which have predominantly occurred in Queensland (Figure 3). Since 2010, there have been more reported dengue cases in other states. However, these are often linked to a traveller returning from an overseas trip with a dengue infection. Queensland is the only state where the *Ae. aegypti* mosquito is present. Other states and territories do not have the *Ae. aegypti* mosquito, so they do not have any local transmission or dengue outbreaks.

27. There are national guidelines for public health units and frameworks for the surveillance and control of dengue virus infection in Australia (Communicable Diseases Network Australia, 2015; National Arbovirus and Malaria Advisory Committee, 2015). These guidelines include the surveillance and response to the disease in humans; and the surveillance of mosquitoes that could carry the disease. The Queensland

government also has surveillance programs in place to detect numbers of mosquitoes and disease cases . (Queensland Health, 2015).



**Figure 3 Frequency of reported cases (locally and overseas acquired) of dengue virus infection in Australia (1991 – 2025) (Department of Health and Aged Care, 2025).**

28. The risk-benefit analysis of the deployment of the proposed GM mosquitoes and the comparison to already existing control methods, including *Wolbachia*-carrying *Ae. aegypti*, does not fall within the scope of the *Gene Technology Act 2000*, which aims to protect the health and safety of people and to protect the environment.

*Aedes mosquitoes can transmit arboviral diseases. Only females transmit disease as males don't blood feed. Dengue fever is predominantly reported in Queensland and often linked to returned travellers. Australia has vector control measures and surveillance programs in place to control and monitor mosquito borne diseases.*

## Section 2 The proposed dealings

29. *Aedes aegypti* is a major insect vector that carries and transmits arboviruses such as yellow fever virus, dengue virus, zika virus and chikungunya virus and can be found in northern Queensland, and in some locations in central and southern Queensland and the Torres Strait. *Ae. aegypti* is not present in southeast Queensland population centres.

30. Oxitec Australia Pty Ltd (Oxitec) is seeking authorisation for the commercial supply of GM *Ae. aegypti* (OX3054) to control the population of the mosquito and prevent the spread of dengue virus in Queensland. They have indicated that, if *Ae. aegypti* colonise other States and Territories in the future, they may apply for the release of the GM mosquito to these areas.

31. For the ongoing commercial supply of the GM mosquito, the dealings assessed by the Regulator are to:

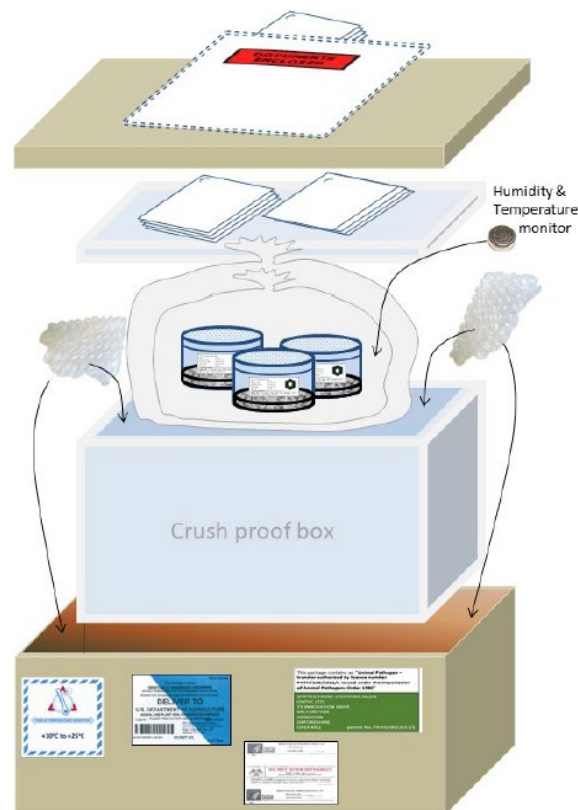
- (a) import the GM mosquito;
- (b) transport the GM mosquito;
- (c) conduct experiment with the GM mosquito (collect and analyse samples and perform demonstrations of the technology);
- (d) grow, raise or culture the GM mosquito;

(e) dispose of the GM mosquito;

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

## 2.1 Details of the proposed dealings

32. The rearing of the GM mosquitoes would be carried out overseas in a dedicated insect rearing facility in the United Kingdom (UK). Mosquitoes are reared in containment suites to ensure separation from other mosquito species; and batch tested to ensure that the batch contains the required number of GM mosquitoes and no arboviruses are present. Mosquito eggs would be collected, dried and imported as egg aliquots or already packaged into OX3054 rechargers (rechargers are placed into mosquito rearing boxes described below). The egg aliquots or rechargers will be packaged in triple or double containment respectively for import into Australia (Figure 4). The eggs are non-motile life stages of the GM mosquitoes and can remain viable as “dried” eggs for several months.



**Figure 4 Packaging process of OX5034 eggs for shipment.** Eggs or OX5034 rechargers will be packaged into a polythene bag, placed into a shatter proof container, then into a crush proof box that is insulated (usually made of expanded polystyrene) and placed into a cardboard box labelled with the shipping conditions.

33. OX5034 rechargers would be assembled into mosquito boxes in a dedicated Oxitec Australia facility based in Queensland (Figure 5). Smaller single use boxes (cardboard) with an egg hatching vessel may also be used depending on the commercial demands of customers. The box would be labelled “**To use in Queensland only**”.





**Figure 5 Typical mosquito rearing boxes.**

34. Within Australia, the mosquito eggs would be transported within the mosquito boxes under temperature-controlled conditions (Figure 5). Water is added to the mosquito boxes upon deployment in the field and male OX5034 mosquitoes are expected to exit the boxes and breed with the local population of *Ae. aegypti*.

35. It is expected that what remains in the mosquito boxes would be mostly dead female larvae and debris from hatched adults. The applicant has proposed that any water remaining in the box would be disposed of by pouring it on the ground or onto the soil of a pot plant.

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*GM mosquitoes will be grown and tested for arboviruses before the eggs are dried and imported into Australia. The boxes containing the GM mosquitoes will be assembled in Australia before use. The GM mosquitoes are only to be used in Queensland.*

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## Section 3 Parent organism

36. The GM mosquito is derived from a strain of *Ae. aegypti*. The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with the GM mosquito. As such, the relevant biological properties of *Ae. aegypti* will be discussed here.

### 3.1 *Aedes aegypti*

37. The mosquito *Ae. aegypti*, commonly known as the yellow fever virus mosquito, is an arthropod that belongs to the family Culicidae that was first described by Linnaeus in 1762 (OECD, 2018). Three subspecies were initially identified, *Ae. aegypti formosus*, *Ae. aegypti aegypti* and *Ae. aegypti queenslandensis* (Powell and Tabachnick, 2013). However, more recent analysis suggests that, *Ae. aegypti queenslandensis* and *Ae. aegypti aegypti* are genomically identical (Rasic et al., 2016).

38. The subspecies *Ae. aegypti formosus* is thought to be the ancestor of the domestic form of *Ae. aegypti*. It is only found in forests and in the transitional area between two forms of vegetation of sub-Saharan Africa and West Africa (Moore et al., 2013; Powell and Tabachnick, 2013; OECD, 2018).

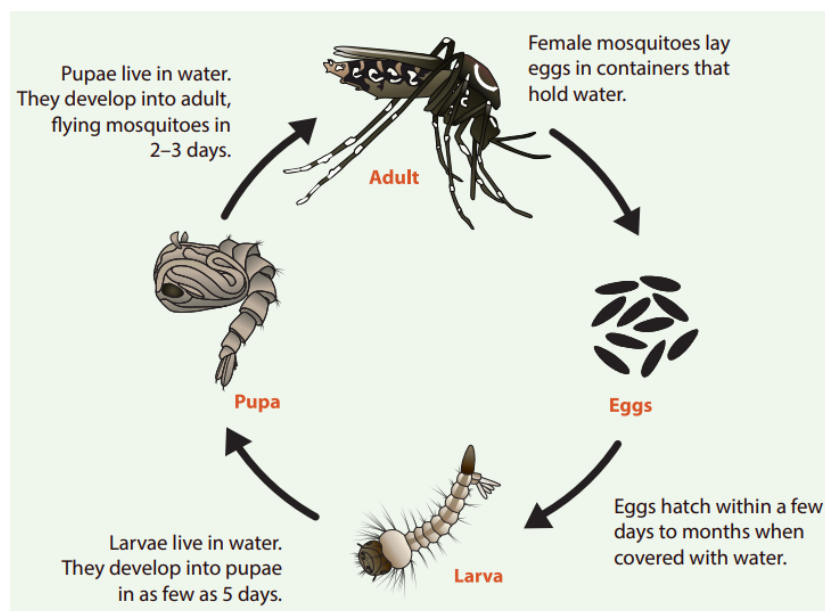
*Ae. aegypti formosus* has been described to prefer laying eggs in tree holes; preferring non-human blood;

and are darker in colour compared to its descendants *Ae. aegypti aegypti* (also more commonly known as *Ae. aegypti*) (Moore et al., 2013; Powell and Tabachnick, 2013; OECD, 2018).

39. *Ae. aegypti* unlike its ancestor can be found in tropical and subtropical regions worldwide but is absent from the interior of Africa and south of the Sahara. Unlike many mosquito species, for which their preferential habitat consists of bushland or swamps, *Ae. aegypti* breeds indoors or in artificial containers outside houses, and are typically found in association with humans (Moore et al., 2013; Powell and Tabachnick, 2013; OECD, 2018).

40. Their association with humans is likely because they almost exclusively feed on humans (99%) and in low frequencies (<1%) on other hosts (e.g. bovine, swine, cat, rat, and chicken) (Ponlawat and Harrington, 2005). A more recent Australian study of mosquitoes in Cairns demonstrated that approximately 75% of *Ae. aegypti* tested (n = 174) had a blood meal from humans and approximately 19% had a blood meal from dogs and birds (Jansen et al., 2009). Both males and females can feed on plant sugar sources such as nectar but males feed exclusively on plant sugar sources and are not known to feed on blood (Olson et al., 2020).

41. The *Ae. aegypti* lifecycle involves four distinct stages and is dependent on the presence of water and ambient temperature (OECD, 2018). In brief, female mosquitoes lay their eggs in containers that hold water. The eggs then hatch into larvae, which develop into pupae before the mosquitoes finally emerge as flying adults (Figure 6) (US Centers for Disease Control and Prevention, 2022). The eggs, larva and pupa are all aquatic stages of the life cycle.



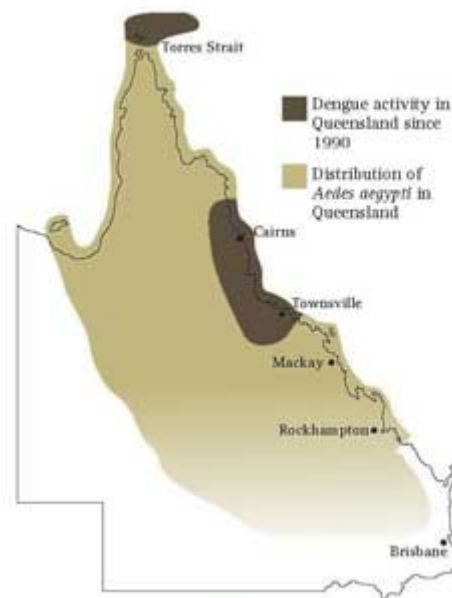
**Figure 6 Life cycle of *Ae. aegypti* mosquitoes (US Centers for Disease Control and Prevention, 2022).** Egg hatches into a larva, which develops into a pupa in the water before emerging as an adult mosquito within 7-10 days.

42. The typical reported life expectancy of female adults in the wild ranges from 10-35 days and 3-6 days for male mosquitoes and is dependent on temperature (i.e. shorter in tropical regions and longer in more temperate climates) (OECD, 2018).

43. Previous studies conducted in Northern Queensland estimated that, *Ae. aegypti* males have an average life expectancy (ALE) of between 0.64 and 1.69 days and can travel an average distance of 35m and up to a maximum of 297m (Muir and Kay, 1998; Russell et al., 2005; Trewin et al., 2021). Females have an ALE of 0.86 days and travel an average distance of between 56 – 77m (Muir and Kay, 1998; Russell et al., 2005).



44. *Ae. aegypti* is not native to Australia and was introduced over 100 years ago. Currently, it is established in northern Queensland, and in some locations in central and southern Queensland (Figure 7) (Queensland Government, 2021). However, in the past, it was found across Queensland, in parts of the Northern Territory and northern New South Wales (Trewin et al., 2017; Queensland Government, 2021).



**Figure 7 Distribution of *Ae. aegypti* in Australia (Queensland Government, 2021).**

45. The eradication of *Ae. aegypti* in Brisbane was attributed to the combination of public education and government public health responses (Trewin et al., 2017). Other methods that have been used in the state of Queensland to manage the population of *Ae. aegypti* and control dengue, includes the release of mosquitoes containing the endosymbiont *Wolbachia* (Beebe et al., 2021; Ogunlade et al., 2023; CSIRO, 2024).

46. Natural predators of *Ae. aegypti* feed on both aquatic (larvae and pupae) and adult stages of the mosquito. Predators of the aquatic stages include fish, early stages of odonates (dragonflies and damselflies), other mosquito species (e.g. *Toxorhynchites*; elephant mosquitoes), amphibians, tadpoles and copepods (small crustaceans) (OECD, 2018; Bimbile Somda et al., 2022). Predators that target adult stages include bats, adult odonates, amphibians (frogs, geckos), birds, arthropods (mantis, small flying insects) and spiders (OECD, 2018; Bimbile Somda et al., 2022). However, none of these species have been shown to be solely reliant on *Ae. aegypti* as their primary food source (OECD, 2018).

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*Ae. aegypti* is present in some parts of Queensland. They have a short lifespan and do not fly long distances. They live in association with people in and around houses. Only female mosquitoes bite. No species are solely reliant on *Ae. aegypti* as a primary food source.

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### 3.2 *Aedes aegypti* as a mosquito vector for disease

47. *Aedes aegypti* mosquitoes are one of the main insect vectors that transmit mosquito borne viruses to humans such as dengue virus, Zika virus, chikungunya virus and yellow fever virus (Ogunlade et al., 2021). There are various other arboviruses that are present in Australia that can cause disease such as Barmah Forest virus, Ross River virus, Murray Valley encephalitis virus, West Nile virus Kunjin strain and

Japanese encephalitis virus. However, *Ae. aegypti* is not known to be a primary vector for the transmission of these viruses (Viennet et al., 2024).

48. Laboratory studies have also suggested that, *Ae. aegypti* may be a vector for lumpy skin disease virus (Chihota et al., 2001; Riana et al., 2024), a disease that infects cattle (Whittle et al., 2023). Lumpy skin disease is currently not present in Australia (Department of Agriculture Fisheries and Forestry, 2023a). However, no data are available confirming that, *Ae. aegypti* can transmit lumpy skin disease outside of a laboratory setting.

49. Mosquitoes are infected by these viruses through feeding on the blood of infected people and transmitting the virus to uninfected people through the same mechanism (Mack, 2016; Huang et al., 2019). Only females transmit disease as males do not blood feed (Mack, 2016; Huang et al., 2019).

50. The mosquito microbiome (collection of microorganisms such as bacteria, virus, fungi, protozoa which live together), has been suggested as one of the many factors that contribute to the capacity of mosquitoes to transmit arboviruses. The environment where the mosquito population originates plays an important role in the composition of the microbiome of the mosquito. The differences in the microbiome could increase or decrease the potential for transmission of the diseases (vector competence), however, it is still unclear whether it plays a main role in vector competence. The impacts of the microbiome to transmit arboviruses in *Ae. aegypti* has been extensively reviewed (Scolari et al., 2019; Souza-Neto et al., 2019; Ferreira et al., 2023). It is likely that the mosquito strain used to generate the GMO has a different microbiome to the native wild type (WT) strain in Australia and so could potentially have a different vector competence compared to the native WT strain in Australia.

51. Laboratory strains of *Ae. aegypti* have also been shown to be less genetically diverse than the mosquitoes present in the wild, which could impact on vector competence (Gloria-Soria et al., 2019).

### 3.3 Strain used for generating the GM mosquitoes

52. The parent strain used to generate the GM mosquitoes is a genetically diverse laboratory strain of *Ae. aegypti* originally collected in several regions of Chiapas, Mexico. This strain was transferred from Mexico's Institute of Public Health to Oxitec's United Kingdom (UK) laboratories in 2006, where a continuous colony has been maintained (Spinner et al., 2022). The parent strain is referred to in this RARMP as 'Latin wild type'.

53. Bioinformatics studies demonstrated that although very similar, there are genetic differences between the South American strain and the currently endemic Australian strain, which are more closely related to strains found in Asia (Gloria-Soria et al., 2016). The applicant has stated they are using this Mexican strain rather than a local Australian strain, mainly because it has been extensively characterised and has a known insecticide susceptibility profile.

## Section 4 The GMO - nature and effect of the genetic modification

### 4.1 The genetic modifications

54. The GM mosquito is modified to express a fluorescent marker and a recombinant protein that is regulated by a female specific splicing module. The details of the genetic modifications are summarised in Table 1 and 2 and further described in Sections 4.1.1 to 4.1.4 below.

**Table 1 Introduced genes in the GM mosquito**

Gene	Source organism	Function	Effect
<i>DsRed2</i>	Coral ( <i>Discosoma spp</i> )	Encodes red fluorescent protein.	Mosquito glows red under specialised lights in laboratory.

tTAV	Synthetic fusion tetracycline transactivator protein ( <i>Escherichia coli</i> and <i>Herpes simplex virus</i> )	Encodes tetracycline-repressible transcription factor.	Overexpression of tTVA leads to death of larvae.
Aeadsx splicing module	<i>Ae. aegypti</i>	Allows female specific expression of tTAV.	tTAV only overexpressed in female mosquitoes – so only females die at larval stage.

**Table 2 Other introduced sequences in GM mosquito**

Genetic element	Source organism	Function
<i>TetO x 7</i>	Synthetic DNA containing 7 repeats of tet-operon	Binds to tTAV in the absence of tetracycline, allowing the expression of neighbouring promoter.
<i>Ubiquitin</i>	Fruit fly ( <i>Drosophila melanogaster</i> )	Stimulates cleavage of tTAV protein.
Scraps intron and exonic fragments	Fruit fly ( <i>Drosophila melanogaster</i> )	Intron cloned upstream of DsRed2 coding sequence to facilitate transcription of mRNA.
<i>nls</i>	Synthetic sequence	Nuclear localisation signal for the import of DsRed2 into cell nucleus.
<i>piggyBac 5' and 3'</i>	Synthetic based on cabbage looper ( <i>Trichoplusia ni</i> )	Facilitates the insertion of the recombinant DNA in the presence of piggyBac transposase.
<i>HR5</i>	<i>Autographa californica Nucleopolyhedrovirus (AcNPV)</i>	Transcriptional enhancer to stimulate expression from IE1 promoter.
<i>IE1</i>	<i>AcNPV</i>	Promoter to drive expression of DsRed2.
<i>SV40 3' UTR</i>	<i>Simian virus (SV40)</i>	Transcription termination and polyadenylation site.
<i>DmHsp70 minipro and 5'UTR</i>	Fruit fly ( <i>Drosophila melanogaster</i> )	Promoter to drive the expression of tTAV when tTAV is bound to TetO operator.

**4.1.1 Conditional female-specific self-limiting trait**

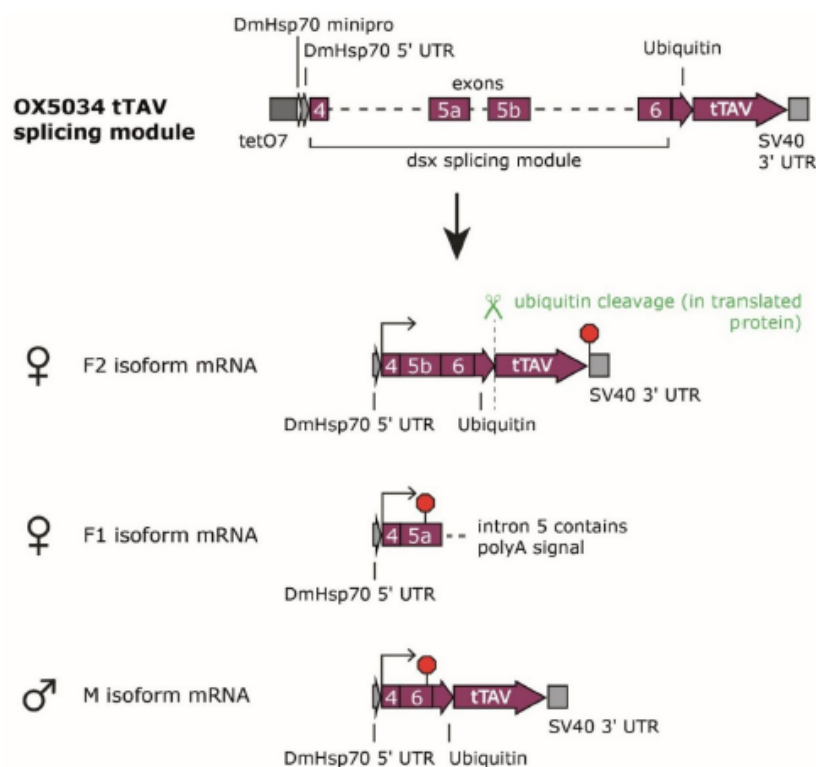
55. The GM mosquito is designed so that female larvae do not survive into adulthood resulting in only adult males emerging.

56. Female larval death is due to the female specific expression of a tTAV-OX5034 (tTAV) protein, which is a variant of the transactivator protein (tTA), originally described in *Escherichia coli* (Gossen and Bujard, 1992), whereby the tetracycline repressor (tetR) is fused to a segment of a *Herpes simplex virus* VP16 protein. The mechanisms of the female specific expression of tTAV and how it prevents GM female mosquitoes from reaching adulthood are further described below.

57. The expression of tTAV is under the control of a sex specific splicing module derived from the *Ae. aegypti doublesex* (*Aeadsx*) gene. The *Aeadsx* splice module consists of exons 4, 5a, 5b and 6; and introns 4, 5 and 6 (Figure 8).

58. Sequences encoding seven tetracycline operators (TetO7), and the *Drosophila melanogaster* heat shock protein 70 (*DmHsp70*) are located upstream of the splice module. Sequences encoding ubiquitin, tTAV and SV40 (transcription termination sequence and polyadenylation signal) are downstream of the splicing module (Figure 8).

59. In GM females, two different isoforms of mRNA are transcribed (F1 and F2) from the splicing module but only the isoform F2 leads to the expression of the tTAV protein. A stop codon leads to a shorter mRNA transcribed in GM males (M) and prevents the production of tTAV protein (Figure 8).

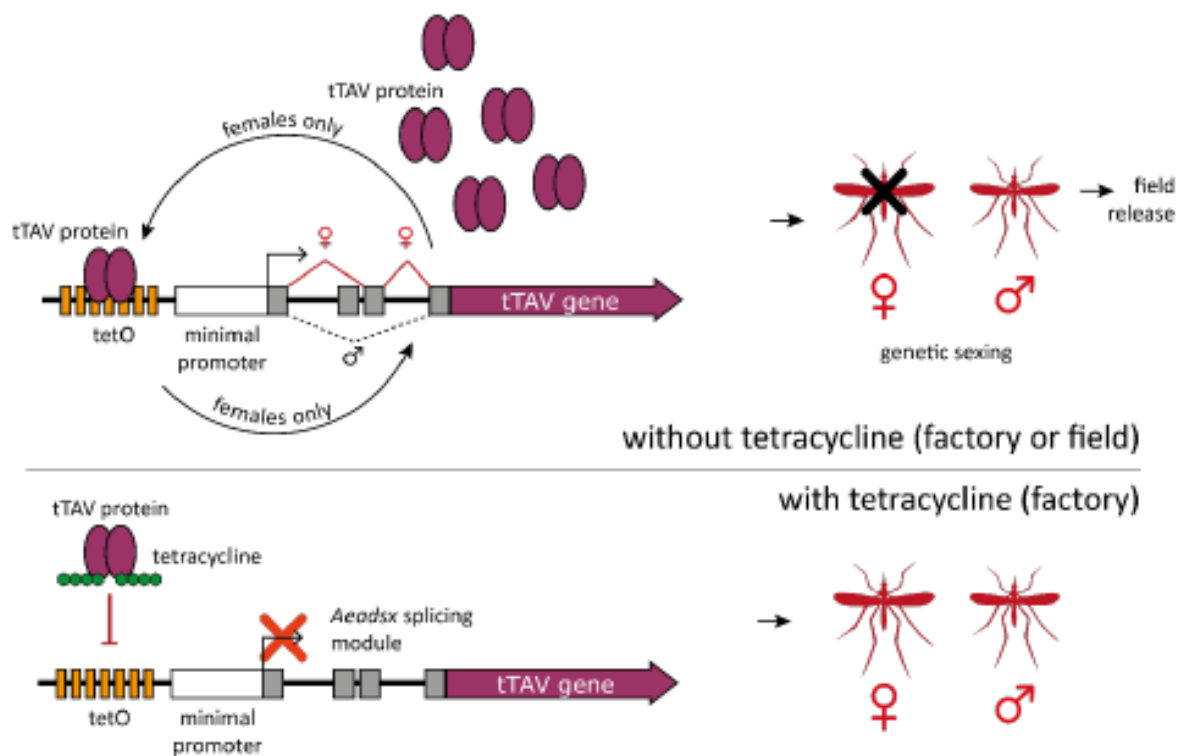


**Figure 8** *Aeadsx* splice module and different isoforms of mRNA transcribed in female and male OX5034. Exons are indicated by maroon boxes and introns are indicated by dashed horizontal lines.

60. In GM females, the expressed tTAV protein binds to the introduced TetO binding sites that drives the minimal *DmHsp70* promoter, resulting in the expression of tTAV following cleavage at the ubiquitin cleavage site. This creates a positive feedback loop that further enhances the expression of tTAV (Figure 9).

61. The mode of action is proposed to involve the overexpression of tTAV protein leading to female lethality due to the inability of the cells to produce other proteins needed to function normally (transcriptional squelching) (Spinner et al., 2022).

62. In the presence of tetracycline or its derivatives, female GM mosquitoes survive into adulthood because tTAV is unable to bind to the TetO binding sites and tTAV protein is not continuously expressed in GM females, (Figure 9). This principle is used during the manufacture of the eggs to be commercially supplied.



**Figure 9 Schematic representation of the tTAV system in the absence and presence of tetracycline.**

63. Mosquitoes are bred in the presence of tetracycline during the production of eggs in the UK to enable production of both female and male eggs.

64. The tetracycline inducible system has a history of safe use in mammalian cell culture, bacterial expression systems and animal models (Gallia and Khalili, 1998; Zhu et al., 2002; Stieger et al., 2009; Evans et al., 2019).

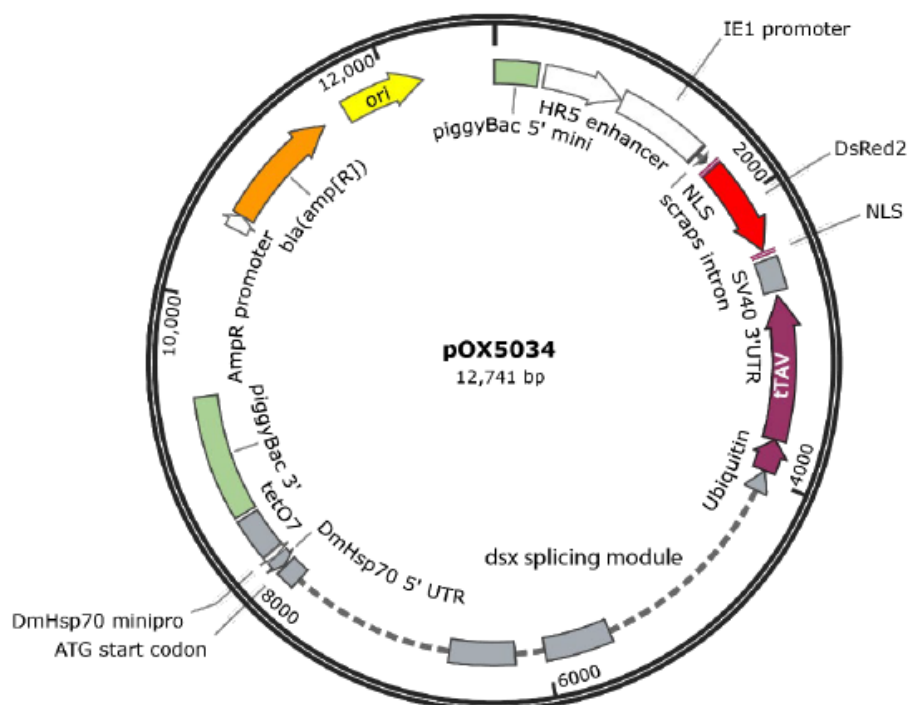
#### 4.1.2 Fluorescent marker

65. The GM mosquitoes (males and females) also express a fluorescent marker DsRed2 (Nishizawa et al., 2006), which is a derivative of DsRed originally isolated from various coral (*Discosoma spp*) (Matz et al., 1999). The expression of DsRed2 is controlled by the *HRS-IE1* enhancer and promoter, which is derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV) and fused to two nuclear localisation sequences (NLS), which helps localise DsRed2 in the nucleus. The expression of DsRed2 in the GM mosquito is independent from the expression of the tTAV cassette and is expected to be produced at every life stage of the mosquito, but predominantly during the larval stage.

66. The expression of DsRed2 can be used to differentiate OX3054 larvae and mosquitoes (males and females, homozygous and hemizygous) from WT under fluorescent light.

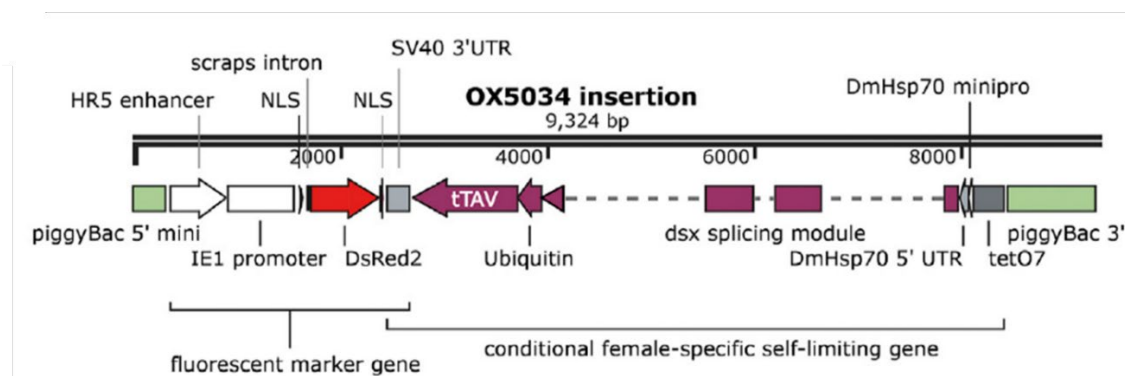
#### 4.1.3 Overview of the recombinant construct/plasmid

67. Both the conditional self-limiting and fluorescent genes are flanked by a non-autonomous transposon inverted terminal repeat sequences from the *Trichoplusia ni* (piggyBac) and cloned into a standard backbone plasmid vector containing ampicillin resistant gene (*ampR*) and bacterial origin of replication (*pUC ori*) to allow for plasmid growth in *E. coli* (Figure 10).



**Figure 10** Plasmid map of pOX5034.

68. In the presence of piggyBac transposase, the recombinant DNA (rDNA) construct between the piggyBac sequence can be integrated into the germline of *Ae. aegypti* (Figure 11). The rest of the plasmid backbone containing the *ampR* and *pUC ori* genes are not integrated into the genome of the mosquitoes. Non-autonomous transposons do not encode elements of transposase or reverse transcriptase, which is needed for the transposons to translocate. Therefore, they must rely on these proteins from other sources for translocation to occur (Pray, 2008).



**Figure 11** Recombinant DNA construct inserted into mosquito genome (Spinner et al., 2022).

#### 4.1.4 Generation of the OX5034 strain

69. To generate the GM mosquitoes, the plasmid pOX5034 is microinjected into mosquito embryos (Jasinskiene et al., 1998) with a non-integrating source of piggyBac transposase. The presence of the transposase allows for integration of the rDNA construct into the germline cells and excludes the antibiotic resistance and bacterial origin genes (Figure 11). Mosquitoes in which a successful integration of the rDNA occurred can be identified by the fluorescent marker.

70. Multiple crosses of the GM mosquitoes were carried out to create a strain homozygous for the inserted gene.

71. Subsequent sequencing analysis showed that the rDNA is inserted into the intronic sequence of predicted gene AAEL009706 on chromosome 3 based on the genome assembly for *Ae. aegypti* (AaegL3 and AaefL5) (Spinner et al., 2022), meaning that no genes were disrupted by this insertion.

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*The GM mosquito is modified to ensure only male offspring survive following mating with wild type mosquitoes. The GM mosquitoes also express a fluorescent marker for identification under specialised light. No other mosquito genes were disrupted by the insertion of the gene cassette. No antibiotic resistance genes are inserted into the GM mosquitoes.*

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## 4.2 Characterisation of the GM mosquito

### 4.2.1 Longevity and bio-distribution

72. Laboratory data demonstrated no difference in the survival of homozygous male GM mosquitoes (carries two copies of the tTAV transgene, one of which is inherited from each parent) compared to WT when reared in the presence of the tetracycline derivative doxycycline (similar to factory breeding conditions) (Spinner et al., 2022). In contrast, homozygous male GM mosquitoes may have reduced longevity compared to WT in the absence of doxycycline (similar to field release conditions) (Spinner et al., 2022). No differences in survival rates were observed between hemizygous male GM mosquitoes (only carries one copy of the tTAV transgene, which is inherited from a GM parent) compared to WT in the absence of doxycycline (Spinner et al., 2022). Homozygous female GM mosquitoes reared in the presence of doxycycline have a reduced survival rate compared to WT female mosquitoes (Spinner et al., 2022). Homozygous female GM mosquitoes do not survive into adulthood in the absence of doxycycline (Spinner et al., 2022).

73. In field trial studies, in Brazil, male GM mosquitoes have been shown to survive for a maximum of 7 days (mean 1.3 days) and were able to travel up to a maximum of 198m (mean 54.8m) (Spinner et al., 2022). The average lifespan for the GM mosquitoes is not outside the range seen for the wild *Ae. aegypti* populations as described in Section 3.1.

### 4.2.2 Clutch egg sizes

74. The applicant has provided data that demonstrated that the number of eggs laid by WT females (clutch size) mated with homozygous male GM mosquitoes are smaller than those from WT females mated with WT male mosquitoes under laboratory conditions.

75. Other methods of rearing mosquitoes have described clutch sizes of 100-200 eggs/clutch depending on the size of the blood meal, which are larger than the clutch sizes of the GM mosquitoes (Clemons et al., 2010; Masters et al., 2020; Gunara et al., 2023).

### 4.2.3 Expression of fluorescent marker

76. As mentioned in Section 4.1.2, the GM mosquitoes, both male and female, express a fluorescence marker (DsRed2) in the larval and adult stages, which can be identified under fluorescent light. While WT and GM mosquitoes will appear the same in the field, collection of larvae in ovitraps and observation under appropriate lighting/filters will allow to easy identification of GM larvae and mosquitoes from WT.

77. DsRed2 has been extensively used in rats (Murakami and Kobayashi, 2005; Haga et al., 2017), plants (Hu et al., 2022; Uetz et al., 2022; Huai et al., 2023), fungi (Nahalkova and Fatehi, 2003), bacteria (Li et al., 2019) and mammalian cells (Maruyama et al., 2004) as a visualisation tool to differentiate GM organisms from WT organisms.



#### 4.2.4 Penetrance and genetic inheritance of profile

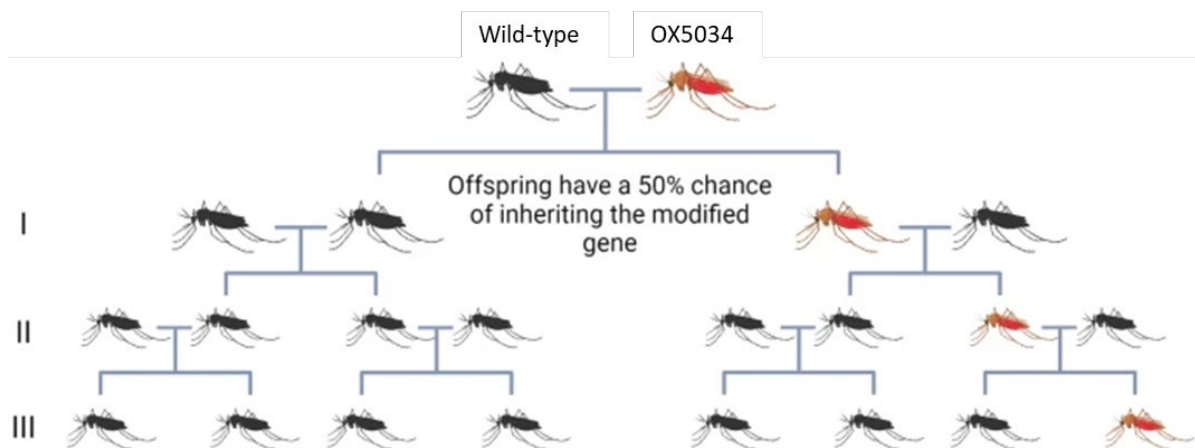
78. Penetrance in genetic terms refers to the proportion of individuals in a population who carry a specific gene and express the related trait. In the case of this GM mosquito, penetrance of the self-limiting trait of the genetic modification refers to the proportion of female mosquitoes that die before reproductive age in the absence of tetracycline or its derivative.

79. Published data demonstrated that in a laboratory setting, both hemizygous and homozygous male GM mosquitoes survive into adulthood in the presence and absence of 4 µg/ml of doxycycline (Spinner et al., 2022). However, no hemizygous and homozygous female GM mosquitoes survive into adulthood without doxycycline (Spinner et al., 2022).

80. Similarly, during field trials in Brazil, eggs laid during the trial were hatched in the laboratory. No females hatched from these eggs (Spinner et al., 2022).

81. The mosquito eggs that are intended to be released would be homozygous for the transgenes. Only adult male GM mosquitoes will hatch from the eggs. When homozygous male GM mosquitoes are released and mate with female WT mosquitoes, all offspring will be hemizygous for the transgene. However, only males would survive into adulthood.

82. These hemizygous male GM mosquitoes will then further mate with WT females and the transgene will pass on to its offspring via normal Mendelian inheritance (Figure 12).



**Figure 12 Mendelian inheritance of genes when hemizygous GM mosquitoes mates with WT females (adapted from (Naidoo and Oliver, 2024)).**

83. Laboratory studies have demonstrated that the the transgene is no longer detected in male GM mosquitoes occurs within a mean of 7.3 ( $\pm 1.2SE$ ) generations with a maximum of 9 generations following consecutive mating of male progeny of each generation with WT female mosquitoes (Spinner et al., 2022). Stochastic modelling has predicted that the male GM mosquitoes carrying the transgene are no longer detected in the environment within 10 generations (Spinner et al., 2022) if no further release occurs.

84. The stability of the Mendelian inheritance of the transgene was measured by crossing homozygous male GM mosquitoes from different generations (G15, G16 and G19 from the same ancestral line) with WT females.

85. The expectation is that the first generation (Cross I; C1) would all inherit the transgene and when males from C1 are further crossed with WT females, it is expected that 50% of the progeny (Cross II; C2) would inherit the transgene and the other 50% would be WT. Data obtained from these crosses showed that C1 all inherited the fluorescent marker as expected and in C2, the ratio of fluorescent to WT larvae was within  $\leq 2\%$  of the expected 50:50 ratio.

86. The applicant has also stated that there has not been any remobilisation of the transgene observed in over 49 generations. This is consistent with current literature that demonstrated that transgenes that



are inserted via piggyBac are unable to translocate to another region in somatic and germline tissue after their initial integration (Sethuraman et al., 2007; O'Brochta et al., 2011; Palavesam et al., 2013).

#### 4.2.5 Molecular characterisation

87. The applicant provided molecular characterisation data using polymerase chain reaction (PCR); genomic sequencing and analysis; and southern blot to demonstrate that:

- (a) the transgene between the *piggyBac* elements is inserted as a single complete copy, without rearrangements in the GM mosquitoes as intended;
- (b) the insertion site of the transgene has been characterised and is unlikely to disrupt any known *Ae. aegypti* protein coding sequences or result in novel open reading frames; and
- (c) the plasmid backbone containing the *ampR* and *pUC ori* genes has not been shown to integrate into the genome of hemizygous male GM mosquitoes.

88. The methods used and a summary of the results are shown in Table 3.

**Table 3 Summary of methods and results of the molecular characterisation studies on the GM mosquitoes.**

Item	Method used	Results
(a)	PCR and Sanger sequencing to compare the integrated transgene compared to the original plasmid construct.	The integrated transgene was found to be identical to the pOX5034 plasmid without rearrangements.
(b)	Degenerate-primer PCR to identify genomic sequence adjacent to the insertion site. Products were gel purified, cloned and sequenced.  Basic Local Alignment Search Tool (BLAST) used to align the identified sequence to <i>Ae. aegypti</i> AaegL3 and AaegL5 genome and assembly.	Transgene was found to be Integrated into the intronic sequence of predicted gene AAEL009706 on chromosome 3 of <i>Ae. aegypti</i> . AAEL009706 codes for a predicted influenza virus NS1A-binding protein.
(c)	PCR amplification of the plasmid backbone and the transgene containing the transgene of interest in DNA isolated from hemizygous male GM mosquitoes.	No plasmid backbone was detected in the genomic DNA isolated from hemizygous male GM mosquitoes.

89. In conclusion, the provided data confirms that the insertion of the genetic material did not disrupt any critical genes, the entire cassette was inserted as intended and no unintended insertions/modifications elsewhere in the genome were detected.

#### 4.2.6 Expression of transgene in larvae, pupae and adult GM mosquitoes

90. Protein quantification data was provided demonstrating the expression profiles of DsRed2 and tTAV proteins in the larvae, pupae and adult males (GM and WT) reared in the absence of doxycycline to mimic the natural environment. Adults were selected at day 1 and 6 post-eclosion (emergence from pupae). Homozygous adult males, and homozygous and hemizygous larvae and pupae were tested.

91. The protein levels of DsRed2 and tTAV are summarised in Table 4 and Table 5, respectively. Note that both proteins were not detected (ND; not detectable) in samples from WT mosquitoes.

**Table 4 Protein detection levels of DsRed in larvae, pupae and adult male GM mosquitoes. ND (not detectable); <LoD (below the level of detection).**

Mosquito sample	Assay LoD	Genotype	DsRed2 level detected
Larvae	6.25ng / larva	Homozygous	Range: ND – 16.7ng Average: 7.7ng $\pm$ 2.4ng (mean $\pm$ SEM, n=6*)
		Hemizygous	ND (< LoD)
Pupae	6.25ng / pupa	Homozygous	Range: 7.3 – 8.4ng Average: 7.9ng $\pm$ 0.4ng (mean $\pm$ SEM, n=2*)
		Hemizygous	ND (< LoD)
Adult	7.5ng / adult	Homozygous (Day 1)	ND (< LoD)
		Homozygous (Day 6)	Range: 17.2 – 48.9ng Average: 35.3 ng $\pm$ SEM, n=4*)

\*Mosquito samples (n) are aliquots of 20 mosquitoes per sample.

92. DsRed2 is detected in all life stages of homozygous GM mosquitoes (larvae, pupae and Day 6 adults) and not detected in hemizygous GM mosquito (larvae and pupae). Note that the average expression of DsRed2 of homozygous GM adult mosquitoes, larvae and pupae is very close to the LoD of the assay. Therefore, it is expected that the levels of DsRed2 expression in hemizygous GM, larvae and pupae would be lower (around half the expression level of homozygous GM mosquitoes). Although DsRed2 cannot be detected via protein detection assays, they can be visually detected in the hemizygous GM adult mosquitoes, larvae or pupae as mentioned in section 4.1.2.

**Table 5 Protein detection levels of tTAV in larvae, pupae and adult male GM mosquitoes. ND (not detectable); <LoD (below the level of detection).**

Mosquito sample	Assay LoD	Genotype	tTAV level detected
Larvae	3.13ng / larva	Homozygous	ND (< LoD)
		Hemizygous	ND (< LoD)
Pupae	0.78ng / pupa	Homozygous	ND (< LoD)
		Hemizygous	ND (< LoD)
Adult	0.39ng / adult	Homozygous (Day 1)	Range: 2.8 – 4.1ng Average: 3.3ng $\pm$ 0.3ng (mean $\pm$ SEM, n=4*)

		Homozygous (Day 6)	Range: 1.8 – 2.7ng Average: 2.2ng $\pm$ 0.2ng (mean $\pm$ SEM, n =3*)
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\*Mosquito samples (n) are aliquots of 20 mosquitoes per sample.

93. Detection of very low levels of tTAV in adult males suggests that the *dsx* sex-specific splicing is not perfectly regulated in a sex specific manner leading to a leaky expression of tTAV in males but this level is not sufficient to have a deleterious effect on adult male survival.

#### 4.2.7 Allergenicity and toxicity of tTAV and DsRed2

94. Both transgene proteins (tTAV and DsRed2) have a history of safe use in various eukaryotes and human cells as described in Sections 4.1.1 and 4.2.3.

95. Acute oral toxicity studies are usually carried out using rodents such as mice or rats (OECD, 2002). The applicant carried out an acute oral toxicity study in mice using the fixed dose method in accordance with the OECD guidelines (OECD, 2002).

96. As mosquitoes only breed in fresh water, oral toxicity in freshwater fish was tested using standard representative species. The recommended freshwater fish species for oral toxicity testing include zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*), carp (*Cyprinus carpio*), Japanese medaka (*Oryzias latipes*), guppy (*Poecilia reticulata*) and bluegill (*Lepomis macrochirus*) (OECD, 2019). Data provided were generated using guppy fish.

97. The applicant has also provided oral toxicity data on other freshwater invertebrates relevant to mosquitoes which includes American signal crayfish (*Pacifastacus leniusculus*) and elephant mosquitoes (*Toxorhynchites rutilus* and *T. brevipalpis*) as they can feed on mosquito larvae.

98. The laboratory data generated under Good Laboratory Practice (GLP) standards are summarised in Table 6, demonstrating that both proteins:

- (a) lack toxicity in mice;
- (b) lack toxicity in non-target model organisms (mosquito/larvae feeding assays); and
- (c) are rapidly digested by digestive enzymes.

**Table 6 Summary of laboratory studies on toxicity and allergenicity of tTAV and DsRed2**

	Tests
Lack of toxicity in mice (protein feeding studies)	<ul style="list-style-type: none"> <li>2000 mg/kg of either tTAV or DsRed2 proteins were administered to mice via oral gavage.</li> <li>The dose administered was equivalent to the ingestion by a 30 g mouse of over 18 million GM mosquitoes for tTAV and around 1.6 million GM mosquitoes for DsRed</li> <li>There were no deaths, adverse clinical conditions or abnormalities observed in the mice.</li> <li>Concluded that the acute lethal dose &gt;2000 mg/kg.</li> </ul>
Lack of toxicity in non-target model organisms (mosquito feeding studies)	GM and WT mosquito larvae were fed to other species to assess the toxicity of the expressed proteins (tTAV and DsRed) in:

	<ul style="list-style-type: none"> <li>• <b><i>Poecilia reticulata</i> (guppy fish)</b> were fed with larvae at roughly 700g larvae/kg diet for 14 consecutive days. <i>Ae. aegypti</i> Latin Wild Type was used as a control and fed to guppy fish in the same fashion. Mortality was measured daily</li> </ul> <p><b>Outcome</b> – No mortality was observed in either fish fed with the GM larvae or the WT larvae.</p> <ul style="list-style-type: none"> <li>• <b><i>Pacifastacus leniusculus</i> (American signal crayfish)</b> – 10 individually-housed American signal crayfish were fed with 700 g larvae/kg diet for 4 days. <i>Ae. aegypti</i> Latin Wild Type was used as a control. Mortality and any adverse effects were recorded 3 and 6 hours after the first meal then daily subsequently.</li> </ul> <p><b>Outcome</b> – no mortality or sub-lethal symptoms were observed.</p> <ul style="list-style-type: none"> <li>• <b><i>Toxorhynchites rutilus</i> and <i>T. brevipalpis</i> (Elephant mosquitoes)</b> are known predators whose larvae feed on other aquatic invertebrates, including mosquito larvae.</li> </ul> <p><i>T. rutilus</i> and <i>T. brevipalpis</i> larvae were fed on 20 GM or WT larvae daily for 4 days. The difference in mortality for the two groups was recorded.</p> <p><b>Outcome</b> – There was no difference in the mortality rate between the groups fed with the GM and WT larvae.</p> <p>A similar experiment was conducted with <i>Toxorhynchites splendens</i> and <i>T. amboinensis</i> in a published study (Nordin et al., 2013) using a slightly different version of the GM mosquito (OX513A – without the sex specific splicing module) but also expressing DsRed2 and tTAV proteins. OX513 included a late-acting lethal genetic system repressed in the presence of tetracycline. It was designed for use in a sterile-insect (SIT) pest control system (Massonnet-Bruneel et al., 2013) where only males were released in the environment. No differences in mortality between the <i>Toxorhynchites</i> larvae fed with WT or GM larvae was observed.</p> <p>OX5382G (GM <i>Spodoptera frugiperda</i> – fall army worm) expressing the tTAV and DsRed proteins and WT fall army worm pupae were fed to:</p> <ul style="list-style-type: none"> <li>• <b><i>Poecilus cupreus</i> (Carabid beetle)</b> are a predator of fall army worms. Five replicates of 6 beetle (3 males and 3 females) were fed either wild type or defrosted GM pupae of the fall army worm (1 pupae/beetle; on day 0, 4, 7 and 10). Assessment of pupa consumption and mortality were made on day 4, 7, 10 and 14.</li> </ul> <p><b>Note</b> – the level of expression of DsRed2 and tTAV per insect weight (larvae and pupae) are similar between the fall army worm and the GM mosquitoes in this application. One exception is that the GM mosquito pupae had about 7 times the expression of DsRed2 per insect weight compared to the GM fall army worm.</p> <p><b>Outcome</b> – no reduction in consumption and survival were observed between the group of beetles fed on the WT or the GM pupae.</p> <ul style="list-style-type: none"> <li>• <b>Bobwhite quail</b>- two groups of 5 quails (1 male and 4 females) were fed daily with three fall army worm larvae either GM or WT for 5</li> </ul>
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	<p>consecutive days. Birds were monitored for any clinical signs or mortality. A post-mortem examination was conducted.</p> <p><b>Outcome</b> – No mortality or any clinical signs were observed between the two groups fed with either the WT or the GM larvae. No negative impacts on survival were observed in these feeding studies in comparison to WT.</p>
Rapidly digested by environmental proteases and human/mammalian digestive enzymes	<p><i>In vitro</i> studies determined that these proteins are readily digested within 5 to 10 minutes (tTAV) and 1 to 5 mins (DsRed2) of exposure to the following:</p> <ul style="list-style-type: none"> <li>• Environmental proteases (proteinase K and subtilisin A); and</li> <li>• Human/mammalian digestive enzyme (Pepsin simulated gastric fluid; SGF). The digestibility of novel proteins in SGF has been correlated to reduced allergenicity and has been used as a tool to predict potential allergenicity of introduced proteins in food as a result of genetic engineering (Astwood et al., 1996; Herman et al., 2005).</li> </ul>

99. The applicant has also provided bioinformatics data in accordance with the Codex Alimentarius Guidelines (Codex Alimentarius Commission, 2003, 2008; Codex Alimentarius Commission & WHO, 2009) that suggests that these proteins:

- are highly unlikely to spontaneously cross cell membranes due to their large size, molecular weight and net negative charge;
- have no potential toxic effects (comparison of tTAV and DsRed2 protein sequences to known toxins using the [NCBI Entrez](#) search tools did not demonstrate any homology to known toxins);
- have no potential allergenic effects (search of tTAV and DsRed2 protein sequences on Allergenonline.org databases such as [Allergen online](#), [COMPARE database](#), [NCBI Entrez](#) did not match any known allergen sequences above the threshold for allergenicity concerns (Codex Alimentarius Commission, 2008); and
- do not contain signal peptides that would allow secretion of the proteins from the cells (prediction using SignalP 6.0 (Teufel et al., 2022) and DeepLoc 2.0 (Thumhuri et al., 2022)).

100. The applicant has also provided quantitative protein assays to demonstrate that the proteins are not glycosylated as glycosylated proteins may have impact on allergenic potential of proteins.

#### **4.2.8 Previous trials using similar GM mosquitoes**

101. Several trials were conducted both in Brazil and in the USA (Florida and Texas) both with a previous version of the GM mosquito (OX5034) and the GM mosquito proposed in this application.

102. The GM mosquitoes (OX5034) were first released in May 2018 in the urban area of Indaiatuba, in Sao Paulo, Brazil, during the wet season when the number of *Ae. aegypti* were typically the highest. Regular releases of GM male mosquitoes were conducted, and a monitoring program was put in place to analyse the effect of the release. Two doses were tested in urban areas, the first doses of 500 mosquitoes/person/week and the second of 100 mosquitoes/person/week between May 2018 and April 2019 (Spinner et al., 2022).

103. The release of these GM mosquitoes resulted in a significant reduction in the number of *Ae. aegypti* present in the release areas, with a maximum population suppression of between 72 and 81 % during the peak mosquito season. Once the trial ended and the releases were stopped, the DsRed, used to identify the GM male mosquito, was no longer detected 13 weeks after the last release in 3 of the 4 release areas, and 24 weeks for the fourth site. For the fourth site, a small number of GM mosquitoes were detected in the following mosquito peak season. It was thought that, as for wild type mosquitoes, eggs can be viable for up to 6 months in dry conditions and that in this instance GM mosquito eggs

survived to the subsequent season. They could not be detected 24 weeks after the last release (National Technical Commission of Biosafety Brazil – note that the approval is in Portuguese). This indicates that in Brazil, even when the deployment of this method involved a large number and frequent introduction of GM male mosquitoes, the GM mosquitoes did not persist in the long term and the cassette containing the transgene slowly disappeared from the environment (Spinner et al., 2022).

104. In the field, the released GM mosquitoes were found to survive for a maximum of 7 days, which is similar to wild type *Ae. aegypti* and 100% of the female progeny tested died before adulthood (Spinner et al., 2022). No adverse effects were identified (Spinner et al., 2022).

105. The United States Environmental Protection Agency (US EPA) have approved pilot field trials in Florida and Texas.

The US EPA risk assessment of the field trial included the evaluation of the penetrance of the female-lethal trait, risks to humans associated with the GM mosquito, risk of introgression and risk to non-target organisms (US Environmental Protection Agency, 2020). Their assessment determined that there were no unreasonable adverse effects to humans or the environment as a result of the experimental permit to release the GM mosquito (US Environmental Protection Agency, 2020).

#### 4.2.9 Insecticide susceptibility and resistance

106. The heavy use of insecticides to control the mosquito population has led to insecticide resistance worldwide (Meier et al., 2022). Insecticides can be used to kill larvae (larvicide) or adults (adulticide). Insecticides are divided into 4 main classes, pyrethroids (e.g. permethrin, deltamethrin), carbamates (e.g. propoxur), organochlorines (e.g. DDT) and organophosphates (e.g. malathion) (WHO, 2022b). The WHO has recommendations on the concentrations and exposure times of insecticide to *Ae. aegypti* to determine susceptibility and resistance (discriminating concentration) (WHO, 2016a, 2022b).

107. Based on the most recent WHO recommendations (WHO, 2022b), mosquitoes are:

- Confirmed resistant if the mortality is <90% (at least 100 mosquitoes tested).
- Possibly resistant if the mortality is ≥90% but <98%. If 2 tests from the same population consistently shows mortality of <98% then resistance is confirmed.
- Susceptible if the mortality is ≥98%.

108. The applicant has provided data demonstrating that the GM mosquito has similar susceptibility to insecticides used for the control of mosquitoes as the parent wild type *Ae. aegypti* used to generate the GM mosquito (Table 7).

**Table 7 Larvicide and insecticide resistance profiles of WT parent and GM mosquitoes.**

Strain	Larvicide / Insecticide	Knockdown (60 minutes)	Mortality (24 hours)
WT parent organism	0.001 mg/ml temephos	N/A	99% (n=100)
	0.75% permethrin	100% (n=100)	100% (n=100)
	0.05% deltamethrin	100% (n=100)	100% (n=100)
	0.1% propoxur	92% (n=104)	94% (n=104)
	5% malathion	100% (n=100)	100% (n=100)

GM mosquito	0.001 mg/ml temephos	N/A	99% (n=100)
	0.75% permethrin	94% (n=94)	100% (n=94)
	0.05% deltamethrin	98% (n=100)	100% (n=100)
	0.1% propoxur	89% (n=101)	89% (n=101)
	5% malathion	100% (n=91)	100% (n=91)

*Note: The recommended discriminating concentration for temephos recommended by the WHO is 0.012 mg/ml (WHO, 2016a). The discriminating dose of permethrin, deltamethrin, propoxur and malathion were based on the standard operating procedures (SOPs) to test insecticide resistance in Anopheles mosquitoes released by WHO (WHO, 2016b). Since then, WHO has released a new SOP (WHO, 2022b) following a worldwide multicentre study (WHO, 2022a). The new recommendations for Ae. aegypti are 0.4% permethrin, 0.03% deltamethrin, 1.5% malathion and none were available for propoxur (WHO, 2022b). Negative controls were the solvents used for the larvicide and insecticide as.*

109. The larvae of GM and WT mosquito strains are both susceptible to temephos (larvicide). Both GM mosquitoes and WT strains are fully susceptible to insecticides permethrin, deltamethrin and malathion). However, there is a slight resistance to 0.1% Propoxur (94%; WT and 89%; GM mosquito mortality within 24 hours of exposure, respectively).

110. Studies of wild populations of adult female *Ae. aegypti* collected from Townsville in 1995, showed slight resistance to permethrin ( $96.25\% \pm 2.39$  SE at 0.25%), deltamethrin ( $97.5\% \pm 1.44$  SE at 0.025%), and malathion ( $97.5\% \pm 1.44$  SE at 5%); and high resistance to propoxur ( $53.75\% \pm 3.75$  SE female mortality when exposed to 0.1% propoxur) (Canyon and Hii, 1999). However, it is noted that the percentage of permethrin and deltamethrin used in the Canyon and Hill study is lower than the WHO discriminating dose. Insecticide susceptibility testing by Queensland Health and University of Melbourne have indicated no presence of resistance phenotype. The primary pyrethroid used for dengue control in Queensland is lambda-cyhalothrin, which is not tested (personal communication Queensland Health). However, there are other commonly used insecticide that could be used to control the GM mosquitoes.

111. A mutation in the *voltage-gated sodium channel (vgsc)* gene also known as knockdown resistance (*kdr*) has been associated with resistance to type I and II pyrethroids (Schmidt et al., 2024; Uemura et al., 2024). The most common and well documented mutations involved are the valine to isoleucine (or glycine) substitution (V1016I/G) and the phenylalanine to cysteine substitution (F1534C) (Schmidt et al., 2024; Uemura et al., 2024). The applicant has provided data to demonstrate the absence of these mutations in the GM mosquitoes using methods described by Saingamsook *et. al.* (Saingamsook et al., 2017). This insecticide resistant gene is not detected in *Ae. aegypti* in Australia as of 2015 (Endersby-Harshman et al., 2017).

112. Previously, in northern Queensland, selective indoor spraying of the pyrethroid insecticides have been used to control the population of *Ae. aegypti*. Currently, a search of the APVMA's Public Chemical Registration Information System (PubCRIS) showed that chemical insecticide registered for used with mosquitoes belong to the permethrin or pyrethroid group. While propoxur is currently registered in PubCRIS, it is not used for mosquito control.

113. In conclusion, the GM mosquitoes are shown to be susceptible to commonly used insecticides and larvicides (with exception to propoxur, which is not used in Australia for mosquito control); and is not known to carry insecticide resistance genes associated with resistance to type I and II pyrethroids, which are used in Australia for mosquito control.

#### 4.2.10 Compatibility with *Wolbachia*

114. As mentioned in Chapter 1, Section 1.2.1, infection of *Ae aegypti* mosquitoes with the bacteria *Wolbachia* has been successfully used as a method to prevent the transmission of dengue. The applicant has provided information that the *Wolbachia* strains *wMel* and *wAlbB* can be successfully established in the parent strain used to generate the GM mosquitoes. In addition, the information also indicated that *Wolbachia* transmission and virus blocking characteristics were not affected in the parent organism. Therefore, it suggests that *Wolbachia* can be established in the GM mosquitoes; and that the inheritance profile and effectiveness of *Wolbachia* is not impacted.

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*The GM mosquito has very similar characteristics to WT mosquitoes but can be visually identified under specialised fluorescent light. The genetic modification is stable and is passed on to males in each generation. The inherited trait cannot be detected in the population after 10 generations. The proteins expressed are not toxic or allergenic. The GM mosquitoes are susceptible to commonly used insecticides. The effectiveness of Wolbachia is not impacted in the mosquitoes used to make the GMO.*

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## Section 5 The receiving environment

115. The receiving environment forms part of the context for assessing risks associated with dealings with GM mosquitoes (OGTR, 2013). It informs the consideration of potential exposure pathways, including the likelihood of the GM mosquitoes spreading or persisting outside the site of release. Relevant information about the receiving environment includes state and local council requirements relevant to biological pest control agents, and related mosquito species in the environment.

### 5.1 Site of release

116. The GM mosquitoes is intended to be only released in Queensland and may include locations in the Torres Strait Islands (region that separates mainland Australia from Papua New Guinea e.g. Thursday Island) in areas where *Ae. aegypti* is present.

### 5.2 Biosecurity

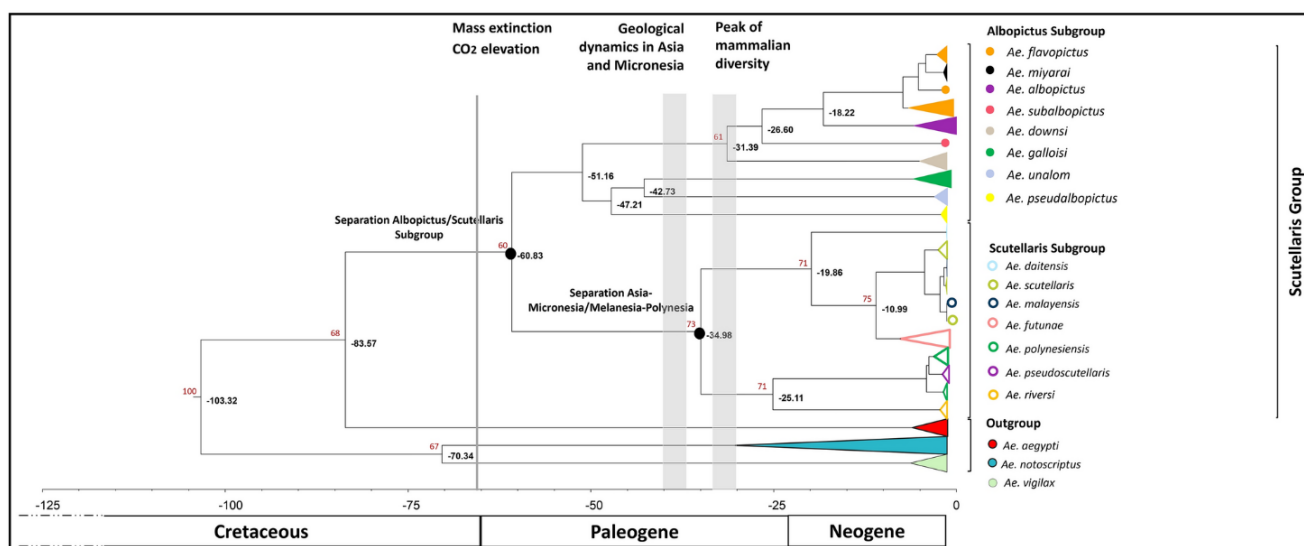
117. As mentioned in Section 1.1, DAFF would be responsible for assessing the national biosecurity risk from import of the GM mosquito and the GM mosquito eggs would need to be included in Part 1 of the Live Import List, which is regulated by DCCEEW.

118. Each state and territory have their own biosecurity regulations and legislation. The Department Primary Industries (QLD) are responsible for leading and coordinating the Queensland Government's biosecurity initiatives.

### 5.3 Presence of related mosquito species in the receiving environment

119. An evolutionary timescale demonstrating the phylogenetic association of the different *Aedes* mosquito species is shown in Figure 13.





**Figure 13 Evolutionary timescale of *Aedes* mosquitoes (Rakotonirina et al., 2024).**

120. Mosquitoes in the subgenus *Stegomyia* have been found in Australia (*Ae. aegypti queenslandensis*, *Ae. albopictus*, and *Ae. scutellaris*) (Webb, 2016). The most common mosquito in Australia (*Ae. notoscriptus*) belongs to the same genus (*Aedes*) but a different subgenus (*Finlaya*) (Webb, 2016). The different mosquito species present in Australia are further described in sections 5.3.1 to 5.3.4 below.

### 5.3.1 *Aedes aegypti* and *Aedes aegypti queenslandensis*

121. As mentioned in section 3.1, *Ae. aegypti* can be found in north and central Queensland and some areas of southern Queensland, which are the potential sites of release.

122. Phylogenetic analysis of *Ae. aegypti* mosquito samples taken from Australia (17 mosquitoes from various areas), Vietnam (15 mosquitoes) and Indonesia (13 mosquitoes) were genomically compared to lineages originating from the regions surrounding Rio De Janerio in Brazil (Gloria-Soria et al., 2016); and in a separate study, a genetic comparison between *Ae. aegypti queenslandensis* with *Ae. aegypti* strains found in Singapore were carried out (Rasic et al., 2016). These phylogenetic studies found that the subspecies *Ae. aegypti queenslandensis* which was originally described in Queensland, Australia is genomically indistinguishable from *Ae. aegypti* (Rasic et al., 2016). Therefore, descriptions of *Ae. aegypti* in Section 3 would also apply to *Ae. aegypti queenslandensis*.

123. *Wolbachia* is an intercellular bacteria shown to suppress the transmission of dengue. Mosquitoes infected with the *Wolbachia wMel* strain were released in north Queensland and have been shown to be able to replace the WT local population (which does not naturally contain *Wolbachia*) (Hoffmann et al., 2011; Hoffmann et al., 2014; Schmidt et al., 2017; O'Neill et al., 2018; Ryan et al., 2019). Although various environmental factors such as temperatures and diet could impact the transmission of *Wolbachia* (Yen and Failloux, 2020), it is expected that the *Ae. aegypti* population in north Queensland, carries *Wolbachia*. *Ae. aegypti* populations in central and southern Queensland are not infected with *Wolbachia*.

### 5.3.2 *Aedes albopictus*

124. *Ae. albopictus* (Asian tiger mosquito) is a highly invasive species that is native to Southeast Asia but has spread around the globe from transport of goods and international travel (Battaglia et al., 2022). Like *Ae. aegypti*, it is responsible for the spread of arboviruses such as yellow fever, dengue, chikungunya, West Nile, Zika and Japanese Encephalitis viruses (Battaglia et al., 2022).

125. The presence of *Ae. albopictus* was first detected in the Torres Strait (in 2005 (van den Hurk et al., 2016). Although surveillance studies have detected *Ae. albopictus* in Australia, there is no evidence that it has established itself in Australia (van den Hurk et al., 2016). However, it is possible that it may establish itself in temperate and tropical areas of Australia in the future (van den Hurk et al., 2016).

126. *Ae. albopictus* and *Ae. aegypti* are distributed in similar habitats as they share similar ecological niches (Bargielowski et al., 2015; Zhou et al., 2022).

127. In the laboratory, mating between *Ae. albopictus* and *Ae. aegypti* have been carried out using “forced artificial” mating (genitalia of opposite sex of anaesthetised mosquitoes are brought into contact) or natural cage mating (where mosquitoes of different species are reared in the same cage) (Lee et al., 2009). In very rare cases interspecies mating can occur in nature (Tripet et al., 2011). However, both laboratory and natural interspecies matings do not result in viable eggs (Lee et al., 2009; Tripet et al., 2011; Bargielowski et al., 2015; van den Hurk et al., 2016; Andrianjakarivony et al., 2022; Zhou et al., 2022).

128. The fact that interspecies matings will produce non-viable eggs has been proposed as a probable cause of competitive displacement of resident mosquitoes by invasive species (satyrisation) when two species of mosquitoes such as *Ae. aegypti* and *Ae. albopictus* are co-located (Bargielowski et al., 2015). Satyrisation occurs when invasive mosquitoes mate with the local population of mosquitoes to limit their expansion because they would not produce viable offspring. However, in areas where *Ae. aegypti* and *Ae. albopictus* co-exist, female *Ae. aegypti* can develop resistance to satyrisation, where females invest more time in mate selection to ensure they do not mate with *Ae. albopictus* and produce non-viable eggs to ensure it is not displaced by *Ae. albopictus* (Bargielowski et al., 2019). Laboratory studies have shown that this resistance is reversible without selection pressure (e.g. presence of *Ae. albopictus*) (Bargielowski et al., 2019). The high cost of satyrisation promotes the avoidance of interspecies mating (Bargielowski et al., 2015).

129. *Ae. albopictus* have been observed to displace *Ae. aegypti* or vice versa depending on various environmental, physical and behavioural factors when the two species are co-located (Bargielowski et al., 2015; van den Hurk et al., 2016; Muzari et al., 2019; Zhou et al., 2022).

130. *Ae. albopictus* naturally carry *Wolbachia* strains *wAlbA* and *wAlbB* but those strains of *Wolbachia* have shown a lesser ability to prevent arbovirus infections in these mosquitoes (Yen and Failloux, 2020).

### 5.3.3 *Aedes scutellaris*

131. *Ae. scutellaris* is mainly found in far north Queensland and the Torres Strait Islands. It has a closer relation to *Ae. albopictus* and has been implicated in as a vector for dengue virus (Moore et al., 2007; Webb, 2016). The larvae have been found in natural and artificial water holding containers (Webb, 2016).

### 5.3.4 *Aedes notoscriptus*

132. *Ae. notoscriptus* (Australian backyard mosquito) is widely distributed in Australia (Pyke et al., 2021). It is a competent vector for canine heartworm and has been implicated in the urban transmission of Ross River and Barmah Forest viruses (Skelton et al., 2016; Pyke et al., 2021; Paris et al., 2023). It has shown varied competency in transmitting other arboviruses that are also carried by *Ae. aegypti* and *Ae. albopictus* (Pyke et al., 2021; Paris et al., 2023).

133. Phylogenetic studies have shown that *Ae. notoscriptus* is less closely related to *Ae. aegypti* compared to *Ae. albopictus* (Cane, 2020). Statistical modelling based on *Ae. notoscriptus* collected in Queensland, suggests that, *Ae. notoscriptus* prefers to fill niches that are not occupied by *Ae. aegypti* (Tun-Lin, 1999). It is unknown whether *Ae. notoscriptus* can interbreed with *Ae. aegypti* to produce fertile offspring. However, if the more closely related *Ae. albopictus* is unable to form a stable hybrid as mentioned in Chapter 5.3.2, it is very unlikely that *Ae. notoscriptus*, which is phylogenetically more distant, can interbreed with *Ae. aegypti* to produce any offspring.

134. Like *Ae. albopictus*, *Ae. notoscriptus* naturally carries *Wolbachia* (*wNoto strain*) (Skelton et al., 2016).

## 5.4 Presence of other mosquito species in the receiving environment

135. There are over 300 mosquito species recorded and widely distributed throughout Australia (Webb, 2016) but only a few pose a significant risk to public health. Mosquito species have evolved to occupy most ecological niches available in Australia (e.g. salt water, fresh water, clean to polluted water, rainforests, estuaries and drought ridden plains) (Webb, 2016).

136. Currently, there are other species of mosquitoes in Australia (*Anopheles spp*, *Ae. vigilax*, *Ae. camptorhynchus*, and *Culex annulirostris*) that are vectors of diseases such as, malaria, Ross River virus, Barmah virus, Murray River encephalitis virus, Kunjin virus and Japanese encephalitis virus (Webb, 2016).

137. Typically, most of these species have habitats such as freshwater sources, (e.g. temporary flooded grassland, rice fields), brackish water, coastal waters, salt marshes, swamps, mangroves, organic rich and often polluted habitats (e.g. open drains, wastewater, sewage ponds and drains), which differ from typical habitats of *Ae. aegypti* (e.g. indoors, in artificial containers in urban areas) (Moore et al., 2013; Powell and Tabachnick, 2013; Webb, 2016).

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*There are other species of Aedes mosquitoes present in Australia. Interbreeding of Ae. aegypti with other species of Aedes mosquitoes do not produce viable offspring. Some Aedes species naturally carry Wolbachia. Ae. aegypti carries Wolbachia that has been introduced into the local population in northern Queensland to control the spread of dengue.*

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## 5.5 Presence of similar genetic material in the environment

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

139. The GM mosquito was derived from an *Ae. aegypti* strain isolated in Mexico and has similar genetic makeup to naturally occurring species found in Australia.

140. DsRed2 and tTAV are isolated from *Discosoma sp.* and *E. coli*, respectively. The tTAV gene is formed from the fusion of tetracycline repressor gene (*tetR*) isolated from *E. coli*, a common bacterium that is widespread in human and animal digestive systems and in the environment in Australia (Gordon and Cowling, 2003) and the transcription factor VP16 from *Herpes simplex virus*, which is also widespread in the human population (James et al., 2020). As such, it is expected that humans, animals and microorganisms routinely encounter the tTAV protein. Coral fluorescent proteins like DsRed are homologous to green fluorescent proteins (GFP) from the jellyfish *Aequorea victoria*, which have been widely used as reporter genes in GM plants (Jach et al., 2001; Alieva et al., 2008; Mann et al., 2012). General information on the use of reporter genes may be found in the document [\*Marker Genes in GM Plants\*](#), also available on the OGTR website.

## Section 6 Previous authorisations

141. The GM mosquito proposed for release has not been approved in Australia but was approved for commercial release in Brazil in 2020.

142. The Brazilian government released a report during this approval process. Part of the justification for the approval of OX5034 in Brazil was that the only organism likely to be affected by this release is *Ae. aegypti*, an invasive species which is already the target of other control methods such as the use of insecticide or other biological methods. No other negative impact was identified (note that the [Brazilian](#)

approval is in Portuguese). As part of this approval, Oxitec proposed to monitor the presence of GM mosquitoes in the environment after the first release. Weekly samples in two representative points in each area of the release will take place until the GM mosquito is no longer detected for four consecutive weeks. Samples will provide information regarding the comparative numbers of WT versus the GM mosquitoes. Once GM mosquitoes have not been detected for four consecutive weeks, monthly monitoring for another four months would take place.

143. Field trials of the GM mosquitoes have been carried out in the USA and Brazil in 2020 and 2018, respectively.

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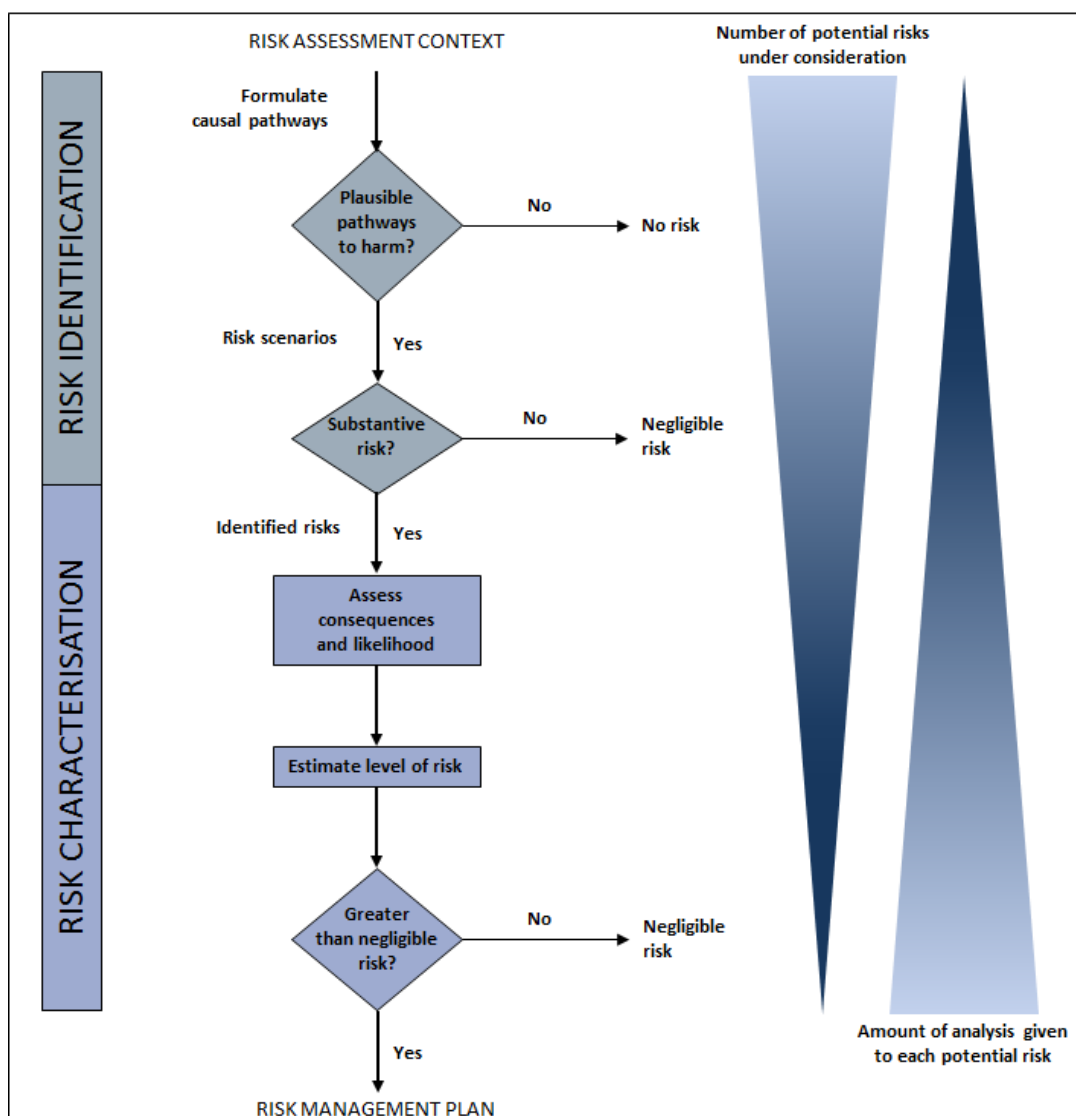
*Genes inserted into the GM mosquitoes are not novel and are already present in the environment. The GM mosquitoes have not been approved for release in Australia. They have been approved in Brazil. Field trials have been carried out in Brazil and USA. No negative impacts have been identified from the commercial release and field trials.*

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## Chapter 2 Risk assessment

### Section 1 Introduction

144. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 14). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.



**Figure 14 The risk assessment process**

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

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

147. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly

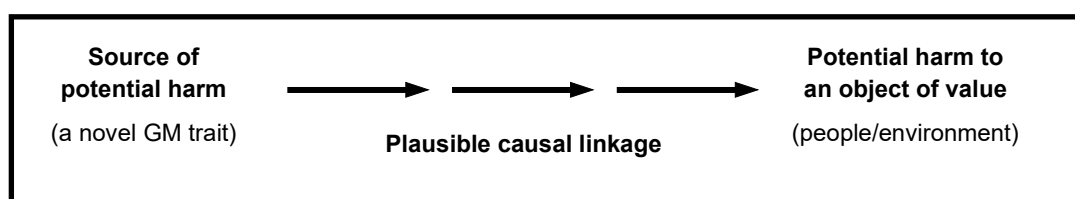
occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 14), i.e. the risk is considered no greater than negligible.

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

## Section 2 Risk identification

149. Postulated risk scenarios are comprised of three components (Figure 15):

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



**Figure 15 Components of a risk scenario**

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

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

### 2.1 Risk source

151. The parent organism is a GM *Ae. aegypti* mosquito expressing a female self-limiting gene and a fluorescent marker. Details of the phenotype and genetic modifications are discussed in Chapter 1.

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

153. As discussed in Chapter 1, Section 4.1, the GM mosquito has been modified by the female specific overexpression of tTAV and a fluorescent protein, including their subsequent offspring. These introduced genes are considered further as a potential risk source.

154. As discussed in Chapter 1, Section 3.2, the mosquito microbiome is one factor that could impact the transmission of arboviruses. Since the unmodified mosquitoes are not from Australia, there may be differences in the microbiome to the *Ae. aegypti* present in Australia. However, as the difference in the microbiome is not a result of gene technology, it is outside the remit of the OGTR and is not considered further as a potential risk. This risk would be considered by other agencies as discussed in Chapter 1, Section 1.1.

155. The current assessment focuses on risks posed to people and to the environment, including long term persistence of the GM mosquitoes, which may arise from the dealings with the GM mosquito.

## 2.2 Causal pathway

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

- the proposed dealings, which are transport or disposal of the GMO and possession (including storage) in the course of any of these dealings,
- regulations for the import and distribution of the GMO by other regulatory agencies, the States and Territories,
- characteristics of the parent organism,
- routes of exposure to the GMOs,
- potential for transmission,
- potential effects of the genetic modification on the properties of the organism,
- potential exposure of other organisms to the GMOs in the environment,
- the release environment,
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential),
- environmental stability of the organism (e.g. tolerance to temperature, UV irradiation and humidity),
- gene transfer to sexually compatible organisms,
- gene transfer by horizontal gene transfer (HGT).
- unauthorised activities.

### 2.2.1 Persistence of GM mosquitoes

157. The presence of tetracycline or a mutation in the female specific splicing cassette in the environment could potentially circumvent the female specific expression of tTAV. This could lead to the survival and potential persistence of the GM mosquitoes in the environment. People or animals could then be potentially exposed to tTAV and a fluorescent protein via bites or ingestion. Therefore, this pathway is further considered as a potential risk source.

158. Similarly, surviving female mosquitoes could potentially be infected and transmit arboviruses. Therefore, this pathway is further considered as a potential risk source.

### 2.2.2 Effects of reduction of *Ae. aegypti* or other mosquitoes

159. The aim of the release of the GM mosquitoes is to reduce the population of *Ae. aegypti*. *Ae. aegypti* is potentially a food source for some animals or insects. A reduction in the population of *Ae. aegypti* could potentially impact other animals or insects that may rely on it as a food source. Therefore, this pathway is further considered as a potential risk source.

160. As mentioned in Chapter 1, section 5.3, there are other species of *Aedes* mosquitoes in Australia. If the GM mosquitoes can interbreed with other *Aedes* species in Australia and transfer the introduced trait, it could lead to the unintentional reduction of other species found in Australia. Therefore, this pathway is further considered as a potential source of risk.

### 2.2.3 Potential of *Ae. aegypti* to transmit lumpy skin disease virus

161. As mentioned in Chapter 1, section 3.2, there is a theoretical possibility of *Ae. aegypti* transmitting lumpy skin disease. However, there has been no evidence of transmission in the field and lumpy skin disease is not endemic to Australia. As only non-biting GM male mosquitoes are expected to survive, they cannot transmit the disease. In addition, *Ae. aegypti* is also usually found in urban areas and not in rural settings where livestock activities usually take place. Therefore, this pathway is not further considered as a potential source of risk.

### **2.2.4 Reduction of *Wolbachia* carrying mosquitoes**

162. The introduction of *Wolbachia* into *Ae. aegypti* populations has been a successful strategy in managing the transmission of dengue in Queensland. As *Wolbachia* is maternally transmitted, the release of the GM mosquitoes could potentially result in the reduction of mosquitoes carrying *Wolbachia* and increase the number of mosquitoes that could be a vector for dengue transmission. Therefore, this pathway is further considered as a potential source of risk.

### **2.2.5 Quality, safety and efficacy of GM mosquito**

163. The APVMA regulates the quality, safety and efficacy, and trade risks associated with the GM mosquitoes under the AgVet Code, as mentioned in Chapter 1, Section 1.1.. Therefore, the quality, safety and efficacy of the GM mosquitoes as a biological pest control agent will not be further considered in this risk assessment.

### **2.2.6 Horizontal gene transfer**

164. Horizontal gene transfer (HGT) is the potential for genetic material to be taken up by an organism. The GM mosquito has been modified to express two proteins, the DsRed and the tTAV proteins. As the GM mosquitoes and larvae die, they would be degraded in soil, water or other media and the genes responsible for encoding those proteins would be present in these environments. The more likely organisms to be able to assimilate those genes into their genome would be micro-organisms. These micro-organisms would have to subsequently transfer these acquired genes to another insect. This pathway is highly unlikely to occur and will not be considered further.

### **2.2.7 Unauthorised activities**

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

## **2.3 Potential harms**

166. Potential harms from the GMO include:

- harm to the health of people, including disease in humans and potential toxicity and allergenicity to the GMO.
- the potential for establishment of GM mosquitoes that could cause harm to the environment.

## **2.4 Postulated risk scenarios**

167. Six risk scenarios were postulated and screened to identify substantive risk. These hypothetical scenarios are summarised in Table 8.

168. In the context of the activities proposed by the applicant and considering both the short and long term, all six risk scenarios did not give rise to any substantive risks that could be greater than negligible (discussed in depth in sections 2.4.1-2.4.6; this chapter).



**Table 8 Summary of hypothetical risk scenarios from dealings with GMO**

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
<b>Risk to health and safety of people</b>					
1	GM mosquitoes	<p>Exposure of eggs/larvae to tetracycline present in environment</p> <p>OR</p> <p>Inactivation of female self-limiting gene via inactivation of the OX5034 cassette</p> <p>↓</p> <p>GM female mosquitoes survive to adulthood.</p> <p>↓</p> <p>GM female mosquito bites a person</p> <p>↓</p> <p>Toxicity or allergic reaction in persons exposed to the tTAV and/or DsRed2 proteins expressed by the mosquitoes</p>	Ill-health in people	No	<ul style="list-style-type: none"> <li>Concentration of tetracycline and its derivatives in the environment is unlikely to be high enough to result in the survival of female GM mosquitoes.</li> <li>Data has shown that the transgenes inserted are stable for 49 generations. Hence, it is unlikely that females can survive and persist in the environment due to a mutation.</li> <li>Transgenes are lost within 10 generations and will not persist in the environment without continuous release of GM mosquitoes.</li> <li>Toxicity feeding studies of tTAV and DsRed2 proteins in mammalian and species that feed on GM mosquitoes did not demonstrate any toxicity.</li> <li>Bioinformatics analysis of tTAV and DsRed2 proteins did not predict any potential toxicity and allergenicity to people.</li> <li>Both DsRed2 and the Tet-Off system has been widely used and did not demonstrate any potential toxicity and allergenicity.</li> </ul>

2	GM mosquitoes	<p>Exposure of eggs/larvae to tetracycline present in environment</p> <p>OR</p> <p>Inactivation of female self-limiting gene via inactivation of the OX5034 cassette</p> <p>↓</p> <p>GM female mosquitoes survive to adulthood</p> <p>↓</p> <p>GM female mosquitoes bite a person infected with an arbovirus</p> <p>↓</p> <p>GM female mosquitoes get infected with arbovirus</p> <p>↓</p> <p>Spread of arbovirus to people via mosquito bites</p>	Increase in the incidence of arbovirus outbreaks	No	<ul style="list-style-type: none"> <li>Females are unlikely to survive and persist in the environment to transmit arboviruses as addressed in risk scenario 1.</li> <li>Low instances of <i>Ae aegypti</i> vectored arbovirus transmission in Australia, reduced the likelihood of mosquitoes acquiring and transmitting an arbovirus.</li> </ul>
3	GM mosquitoes	<p>Release of GM male mosquitoes</p> <p>↓</p> <p>Breeding with <i>Ae. aegypti</i> containing <i>Wolbachia</i></p> <p>↓</p> <p>Reduced population of <i>Ae. aegypti</i> containing <i>Wolbachia</i></p> <p>↓</p>	Increase in the incidence of arbovirus outbreaks	No	<ul style="list-style-type: none"> <li>GM mosquitoes are self-limiting and will not persist in the environment.</li> </ul> <p>Suppression of female survival by the GM mosquitoes would still limit the transmission of arboviruses.</p>

		<p>Reduced effectiveness of <i>Wolbachia</i> as a dengue control strategy</p> <p>↓</p> <p>Spread of arboviruses to people via mosquito bites</p>			
<b>Risk to the environment</b>					
4	GM larvae and mosquitoes	<p>Deployment of the rearing box in the environment</p> <p>↙      ↘</p> <p>Female eggs develop into early larvae and die      Male eggs hatch and develop into adult male mosquitoes</p> <p>↓</p> <p>Animals feed on eggs, larvae, or adult male mosquitoes</p> <p>↓</p> <p>Animals exposed to the tTAV or DsRed protein</p>	Toxicity in animals	No	<ul style="list-style-type: none"> <li>tTAV and DsRed2 are readily digested by environmental proteases or human simulated gastric fluid.</li> <li>Animals and invertebrate feeding studies with GM mosquitoes did not demonstrate any toxicity.</li> </ul>
5	GM mosquitoes	<p>Exposure of eggs/larvae to tetracycline present in environment</p> <p>OR</p> <p>Inactivation of the female self-limiting gene via inactivation of the OX5034 cassette leading</p> <p>↓</p>	Toxicity in animals	No	<ul style="list-style-type: none"> <li>Females are unlikely to survive and persist in the environment to transmit arboviruses as addressed in risk scenario 1.</li> <li>Bioinformatics analysis suggests that the proteins are not secreted by mosquito cells and unlikely to be found in saliva of mosquitoes. Therefore, it is unlikely that</li> </ul>

		<p>GM female mosquitoes survive to adulthood.</p> <p>↓</p> <p>GM female mosquito bites an animal</p> <p>↓</p> <p>Exposure of the animal to tTAV or DsRed protein</p>			<p>these proteins could be transmitted via female biting.</p> <ul style="list-style-type: none"> <li>Exposure of small amounts of tTAV or DsRed2 proteins is unlikely to cause any harm to animals.</li> </ul>
6	GM mosquitoes	<p>Release of GM male mosquitoes</p> <p>↓</p> <p>Breeding with WT <i>Ae. aegypti</i></p> <p>OR</p> <p>Interbreeding with other <i>Aedes</i> species.</p> <p>↓</p> <p>Reduction in population of <i>Ae. aegypti</i> or other closely related species.</p> <p>↓</p> <p>Changes in dynamics of the population of <i>Ae. aegypti</i> or closely related mosquito species.</p>	Imbalance of the ecosystem	No	<ul style="list-style-type: none"> <li>It is highly unlikely that interbreeding with other species of <i>Aedes</i> mosquitoes can result in viable offspring and unlikely to reduce the population of other <i>Aedes spp</i> that are present in Australia.</li> <li>Mosquitoes are unlikely to be a critical food source for any predators in Australia.</li> <li>Insufficient evidence to suggest that, <i>Ae. aegypti</i> is an essential pollinator.</li> </ul>

**2.4.1 Risk scenario 1**

Risk source	GM mosquitoes
Causal pathway	<p>Exposure of eggs/larvae to tetracycline present in environment</p> <p>OR</p> <p>Inactivation of female self-limiting gene via inactivation of the OX5034 cassette</p> <p>↓</p> <p>GM female mosquitoes survive to adulthood.</p> <p>↓</p> <p>GM female mosquito bites a person</p> <p>↓</p> <p>Toxicity or allergic reaction in persons exposed to the tTAV and/or DsRed protein expressed by the mosquitoes</p>
Potential harm	Ill health in people

**Risk source**

169. The source of potential harm for this postulated risk scenario is the GM mosquito.

**Causal Pathway**

170. The exposure of any viable eggs/larvae to tetracycline present in the environment or a mutation in the OX5034 cassette could lead to the female GM mosquitoes surviving into adulthood. The surviving females could then bite a person resulting in exposure to tTAV and the fluorescent protein expressed by the GM mosquitoes via blood feeding. In addition, the surviving females could interbreed with GM or WT male mosquitoes and lead to the persistence of GM mosquitoes in the environment and further exposure of people to the proteins expressed by the GM mosquitoes.

**Presence of significant amount of tetracycline in the environment**

171. Tetracyclines were discovered in the late 1940s and have been used as a broad spectrum antibiotic against various bacterial infections, prophylactically against protozoa, and as a growth promoter in animal feed (Chopra and Roberts, 2001). This class of antibiotic includes tetracycline, doxycycline, minocycline, and two newer drugs tigecycline and eravacycline (Shutter and Akhondi, 2025).

172. Doxycycline is the most common prescribed form of tetracycline and is used as a broad-spectrum antibiotic for the treatment of various types of infections (e.g. syphilis, Lyme disease) but also used for the treatment of acne, or other types of inflammatory skin conditions. This class of antibiotic is also used in animal production systems, such as pigs or chicken, for therapeutic purposes. Its use is however being phased out following a review by APVMA (Langham and Cheng, 2019). Oxytetracycline can also be used in beekeeping to treat European foul brood, a bacterial infection weakening honey bee colonies (Frost, 2021; Agriculture Victoria, 2024).

173. The more likely place where tetracycline may be found in high concentration in Australia would be around water sewage treatment plants, known to accumulate larger concentrations of antibiotics (Akhter et al., 2024). Wastewater analysis has been widely used as a tool to estimate drug use, environmental contaminants and to track prevalence of disease such as severe acute respiratory

syndrome coronavirus 2 (SARS-CoV-2) in the general population (Li et al., 2024). As such, it also a good method to assess the prevalence of antibiotics in the wastewater.

174. The environmental prevalence of tetracycline and its derivatives in the hospital, wastewater treatment plants (WWTP), environmental waters and drinking water in Australia has been carried out in southeast Queensland, Australia between 2005 and 2006 (Table 9A) (Watkinson et al., 2009). More recent data of the prevalence of tetracycline and its derivatives in WWTP throughout Australia (with exception of Western Australia) was carried out in 2021 (Table 9B) (Li et al., 2024).

A	Hospital		WWTP influent		WWTP effluent		Environmental waters		Drinking waters	
	Med	Max	Med	Max	Med	Max	Med	Max	Med	Max
Chlortetracycline	ND	ND	0.006	0.2	0.005	0.25	0.003	0.6	ND	ND
Doxycycline	0.13	0.2	0.02	0.65	0.01	0.15	ND	0.4	ND	ND
Oxytetracycline	ND	ND	ND	0.35	ND	0.07	ND	0.1	ND	ND
Tetracycline	ND	0.04	ND	0.1	ND	0.02	ND	0.08	ND	ND

B	WWTP influent	
	Med	Max
Doxycycline	0.483	2.146
Minocycline	0.106	0.377
Oxytetracycline	0.043	0.143

**Table 9 Concentration of tetracycline and its derivatives in Australia.** Med – median, Max – maximum, ND - not detectable, WWTP – wastewater treatment plant. Concentration is listed as ng/ml. (A) Data from various water sources in southeast Queensland (Watkinson et al., 2009). (B) Data from WWTP throughout Australia (Li et al., 2024).

175. The aquaculture industry also uses oxytetracycline as a preventative measure or to treat bacterial infections and hence may be another environmental source of this antibiotic (Carvalho et al., 2012; Bondad-Reantaso et al., 2023). In Australia, a permit has been granted by the APVMA (PER91309) to use oxytetracycline to treat susceptible bacterial infections in some fish. However, the aquaculture environment is unsuitable for mosquitoes to lay eggs because *Ae. aegypti* prefers to breed in urban areas in small containers that contain fresh water, and it is likely that fish would eat the eggs or larvae.

176. Based on the data that the applicant has provided and the publicly available data on the concentrations of tetracycline and its derivatives in the water systems in Australia, it is highly unlikely that the concentration of tetracycline or its derivatives in the Australian environment would be sufficient to prevent the expression of the tTAV gene and result in the survival of female GM mosquitoes. In the unlikely event that enough tetracycline or its derivatives is present in the environment to allow females GM mosquitoes to develop, the survival of female GM mosquitoes would only be transient as the females would need to continuously lay eggs in water sources containing enough tetracycline or its derivatives to produce viable female GM mosquitoes.

#### Stability of OX5034 cassette

177. The survival of female GM mosquitoes depends on the absence of expression of the tTAV protein. Survival can only occur if either tetracycline is present or the tTAV expression cassette is modified or inactivated, resulting in a different expression profile or absence of this protein.

178. One possible way for down regulation or inactivation to occur might be for transposition of the construct in the mosquito genome to occur with some concomitant change in expression. The expression cassette has been inserted into the mosquito genome using a piggy Bac system. The GM mosquito was produced using a plasmid containing the expression cassette and the delivery of a separate piggy Bac transposase (enzyme). The transposase recognises the two inverted terminal sequence flanking the expression cassette and inserts it in a region of nucleotides sequence TAA within the genome. This insertion is reversible, meaning that if the piggy bac transposase is present, the expression cassette could be remobilised and inserted at a different location within the mosquito genome at a random TAA site. However, the transposase required for remobilisation is no longer

present in the GM mosquitoes and it is therefore highly unlikely that this occurs. Several studies have shown that once a sequence is inserted using the piggy Bac system, remobilisation does not occur (Sethuraman et al., 2007; O'Brochta et al., 2011; Palavesam et al., 2013). In the highly unlikely event that the expression cassette is remobilised, the expression cassette would be inserted at a different region of the mosquito genome, but this would not affect the expression of tTAV and therefore the female GM mosquitoes would still not reach adulthood.

179. A mutation in the promoter of the tTAV expression cassette, the tTAV gene itself or the female specific self-limiting gene could potentially decrease or interrupt the expression of the tTAV protein or result in the expression of the tTAV in both males and females. If the tTAV is no longer expressed, or expressed in smaller amounts, this would allow the survival of female GM mosquitoes. If the tTAV expression is no longer specific to females, all mosquitoes, male and female, would not survive to adulthood. For a mutation to be fixed in a population, it would have to occur in the germ cell lines of a mosquito and be transmitted to its progeny. Mutations occur relatively frequently but persist if they benefit the fitness of the mosquitoes. Ultimately, a mutation would either result in:

- the death of all adult mosquitoes released due to the expression of tTAV in both males and females; or
- a mosquito similar phenotypically to wild type *Ae. aegypti* mosquitoes carrying a non-functional copy of the OX5034 cassette and unable to express the tTAV protein.

180. As mentioned in Section 4.2.4, laboratory data have demonstrated that there has been no remobilisation of the OX5034 cassette for 49 generations of cross mating of the GM mosquitoes with WT. In addition, the survival rate of male mosquitoes (max. 7 days) as described in Section 4.2.1 and the loss of the OX5034 from the population after 10 generations (Spinner et al., 2022) would limit the chances that any mutations can occur and persist.

181. As mentioned in Section 4.2.4, the applicant has provided data regarding the penetrance of the female-specific self-limiting gene (tTAV system). In absence of tetracycline, 100% of the female progeny resulting from the cross between a male or female wild type and a GM mosquito do not survive.

182. Considering the points listed above, it is highly unlikely that either one of these events would occur resulting in female mosquitoes surviving to adulthood.

#### Exposure to the tTAV and the DsRed proteins in people

183. In the unlikely situation where female GM mosquitoes survive to adulthood, the tTAV protein would be either not be expressed at all or expressed at very low levels, not sufficient to result in the death of female larvae. A small amount of the tTAV protein or the DsRed protein may be present when the female GM mosquito bites a person.

184. The applicant has provided data regarding the absence of signal peptides in the GM mosquitoes associated with these proteins. As mentioned in Chapter 1, Section 4.2.7, using bio-informatic tools, no signal peptides were detected. This means that it is highly unlikely that these proteins are secreted by the mosquito cells. Therefore, these proteins are unlikely to be found in the saliva of the mosquitoes and injected into the blood of a person via a mosquito bite.

185. If, however, a small quantity of either protein is transferred to the blood of a person, tTAV protein itself is not toxic (see section 4.2.7). The applicant has provided extensive bioinformatic data supporting the lack of toxicity and allergenicity of these two proteins. This included a literature search, an amino-acid sequences of query proteins and a search through database of known allergenic and toxic substances (Chapter 1 Section 4.2.7).

186. In addition, DsRed2 has been used in other GMOs and its potential toxicity and allergenicity have been investigated in multiple contexts and assessed as safe (Shemiakina et al., 2012; Qureshi and Connolly, 2023).

187. The inducible promoter Tet-Off system also has a history of safe use in eukaryotic cells and animals (Zhu et al., 2002; Munoz et al., 2005; Stieger et al., 2009; Naidoo and Young, 2012; Schönig et al., 2013).

188. Therefore, in the unlikely event that DsRed2 or tTAV is transferred to people or animals, it is unlikely to cause any adverse effects.

### Potential harm

189. Lethality in the female mosquitoes is caused by the disruption of other cellular processes due to the accumulation of the tTAV protein and not the protein itself. The positive feedback loop is responsible for the continuous expression of tTAV protein, thus disrupting the expression of other critical proteins for the development of the mosquito larvae. Exposure to the protein itself would not have the same effect. Therefore, even if a person is exposed to a small amount of tTAV protein is unlikely to cause any harm.

190. The exposure to people to the DsRed2 protein is also unlikely to cause any harm based on the history of safe use in multiple applications discussed above; and the feeding studies and the bioinformatic predictions provided with the application.

### Conclusion

191. The likelihood of harm as a result of exposure of people to proteins via a mosquito bite is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

### 2.4.2 Risk scenario 2

Risk source	GM mosquitoes
Causal pathway	Exposure of eggs/larvae to tetracycline present in environment
	OR
	Inactivation of female self-limiting gene via inactivation of the OX5034 cassette
	↓
	GM female mosquitoes survive to adulthood.
	↓
Potential harm	GM female mosquito bites a person infected with an arbovirus
	↓
	GM female mosquitoes get infected with Arboviruses.
	↓
	Spread of arboviruses to people via mosquito bites
	Increase in mosquitoes that can transmit arboviruses

### Risk Source

192. The source of potential harm for this postulated risk scenario is the GM mosquito.

### Causal Pathway

193. The exposure of any viable eggs/larvae to tetracycline present in the environment or a mutation in the OX5034 cassette could lead to the female mosquitoes surviving into adulthood. The



surviving females could then interbreed with GM or WT mosquitoes and lead to the persistence of the GM mosquitoes in the environment. GM female mosquitoes could potentially serve as a vector carrying arboviruses such as dengue, Zika, chikungunya, and yellow fever viruses and transmit these diseases during a bloodmeal. Male mosquitoes do not blood feed and hence cannot transmit these diseases (Section 3.1). Therefore, if GM female mosquitoes survive and persist, there could be an increased number of mosquitoes able to transmit arboviruses.

#### Survival of GM female mosquitoes

194. As discussed in risk scenario 1, it is highly unlikely that female mosquitoes can survive in the environment.

- The juvenile female mosquito would either have to develop in an environment rich in tetracycline or have to undergo a mutation in the tTAV cassette inactivating the expression of the tTAV protein,
- The applicant has provided data supporting the stability of the expression cassette across 49 generations,
- The applicant has provided data showing 100% penetrance of the female-specific self-limiting gene, meaning it is expected that no adult female mosquito would be released as part of the deployment of rearing boxes.

195. Since only females blood feed and are a vector for transmission of arboviruses, it is highly unlikely that the introduction of GM male mosquitoes can result in the increased transmission of arboviruses such as dengue to people or animals.

#### GM female mosquitoes bite a person infected with an arbovirus

196. Infections caused by arboviruses in Australia are notifiable diseases. As stated in Chapter 1, Section 1.2.2, the cases of dengue, Zika, chikungunya and yellow fever virus infections in Australia are mostly linked to a returned traveller infected with an arbovirus. The most common arbovirus infection in Australia is Ross river virus (mainly transmitted by *Culex annulirostris*, *Aedes vigilax* and *Aedes notoscriptus*), with dengue virus infections (transmitted by *Ae aegypti*) being the second most prevalent. However, local transmission can occur but is low. In 2014-15, out of 1,666 reported cases of dengue in Australia only 74 were locally acquired (Knope et al., 2019). Queensland reported 71 locally acquired cases, while 3 other cases were reported in other states (Knope et al., 2019). Reported outbreaks have almost always been located in Queensland where the vector is present. The absence of a large number of infected persons would limit the likelihood of a GM female mosquito biting an arbovirus-infected person.

#### Spread of arboviruses to people via mosquito bites

197. Spread of the acquired arbovirus by the GM female mosquito would rely on the infected GM mosquito transmitting the disease to another person. The average time required between a mosquito being infected with an arbovirus and the same mosquito being able to transmit the disease via a bite is roughly 8 to 12 days for dengue virus (Scitabile, 2014; Raquin and Lambrechts, 2017). Laboratory data provided by the applicant shows that the life expectancy of homozygous GM female mosquitoes in the presence of doxycycline has a reduced survival rate compared to WT (median survival of 42 and 56 days respectively). Female mosquitoes do not survive into adulthood in the absence of doxycycline. If female GM mosquitoes were to survive, their life expectancy would be around 42 days and therefore they would be able to transmit the disease in a similar fashion to female WT mosquitoes, which typically survive for 10-35 days in the environment (section 3.1).

198. If a GM female mosquito survives, finds a person with an arbovirus infection, gets infected during a blood meal from the arbovirus-infected person and then bites another person, this could result in the spread of the arbovirus infection.

199. The genetic modifications of the GM mosquitoes (expression of tTAV and DsRed2) are unlikely to affect the ability of mosquitoes to transmit arboviruses more efficiently (vector competency) as the modifications have an overall negative impact on the survival of the mosquitoes and aim to only produce male mosquitoes. The vector competency of the parental strain of the GM mosquitoes in comparison to the Australian strains is outside the remit of the OGTR and will be assessed by DAFF, DCCEEW and APVMA.

200. The GM mosquitoes are intended to be released in areas where wild type *Ae. aegypti* are present, as the deployment of the rearing boxes are initially intended to be available in Queensland only. Should a dengue outbreak be triggered as a result of the GM female mosquitoes, it would have to occur in an area where the vector for arboviruses is already present.

201. If the vector is present in the area at levels that support the deployment of GM mosquitoes as a mosquito population control, it is more likely the arbovirus would be spread by local WT mosquitoes, present in much higher concentration than by the surviving GM female mosquitoes released as part of this application.

### Potential harm

202. As mentioned in Chapter 1, Section 1.2.2, arboviruses are national notifiable diseases and there are mosquito surveillance programs in place in Queensland and current pest management strategies would be effective against the GM mosquitoes. As mentioned in Section 4.2.8, the management of an outbreak triggered by the highly unlikely event of a surviving GM female mosquito would not differ from the management of periodic outbreaks in Queensland, consisting for example in the use of insecticides for which both WT and GM mosquitoes are susceptible to.

### Conclusion

203. The increase in mosquitoes that can transmit arboviruses as a result of the release of GM mosquitoes is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

#### 2.4.3 Risk scenario 3

Risk source	GM mosquitoes
Causal pathway	Release of GM male mosquitoes
	↓
	Breeding with <i>Ae. aegypti</i> containing <i>Wolbachia</i>
	↓
	Reduced population of <i>Ae. aegypti</i> containing <i>Wolbachia</i>
	↓
	Reduced effectiveness of <i>Wolbachia</i> as a dengue control strategy
	↓
	Spread of arboviruses to people via mosquito bites
Potential harm	Increase in the incidence of arboviruses outbreak

## Risk Source

204. The source of potential harm for this postulated risk scenario is the GM mosquitoes.

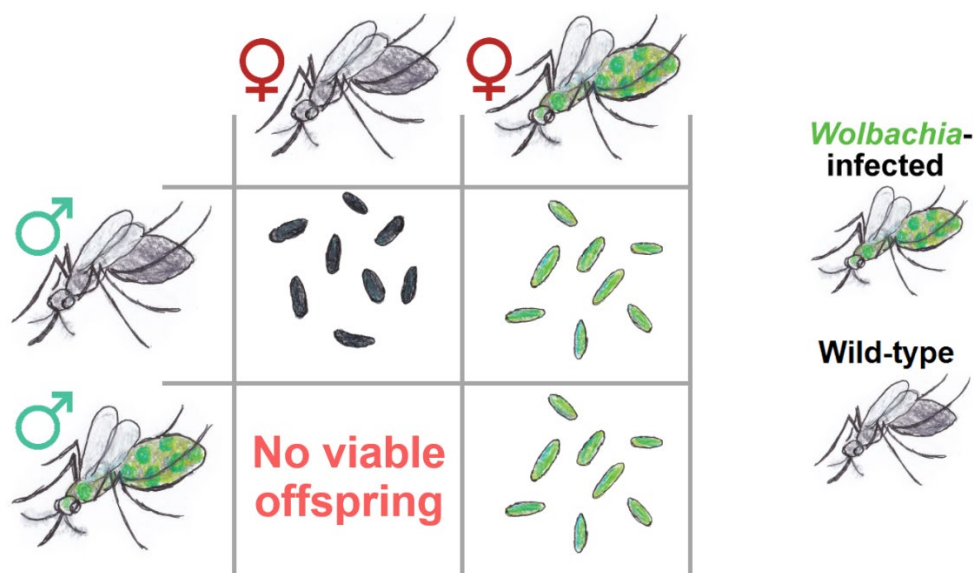
### Causal Pathway

205. As mentioned in Chapter 1, Section 1.2.1, the release of *Ae. aegypti* infected with *Wolbachia* has been a successful biological control strategy in the reduction of dengue outbreaks in northern Queensland by reducing the ability of *Ae. aegypti* to transmit dengue. The main transmission of *Wolbachia* is from infected females to their offspring (Bhattacharyya and Roelke, 2024). The release of the GM mosquitoes is intended to remove female *Ae. aegypti* from the population and hence may indirectly impact on the maintenance of *Wolbachia* in the *Ae. aegypti* population currently present in Queensland. This could potentially result in an increase in mosquito that could transmit dengue.

### Breeding with WT *Ae. aegypti*

206. The only GM mosquitoes released in the environment would be GM male mosquito homozygous for the tTAV and DsRed2 genes. Male GM mosquitoes would then mate with WT female mosquitoes which may be carrying *Wolbachia*.

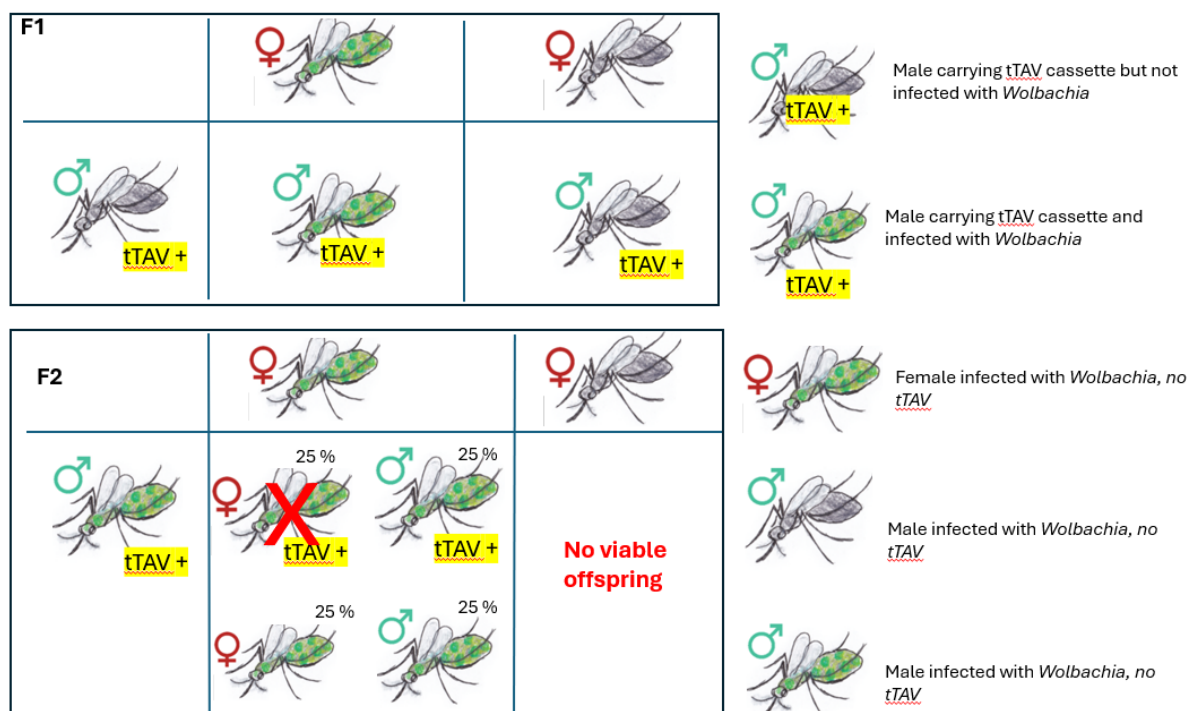
207. *Ae. aegypti* carrying wMel *Wolbachia* has been deployed across areas of northern Queensland where the risk of dengue transmission is higher (Ogunlade et al., 2023). In areas where these releases were conducted a large percentage of *Ae. aegypti* mosquitoes carry *Wolbachia* (over 90 % in 2019). Due to cytoplasmic incompatibility caused by a *Wolbachia*-mediated sperm modification, male mosquitoes carrying *Wolbachia* and mating with a female mosquito not carrying *Wolbachia* do not produce offspring as the eggs resulting from this mating do not hatch. However, male fertility is preserved when mating with females carrying *Wolbachia*, as a factor present in the eggs of these females rescues fertilisation (Figure 16). *Wolbachia* is therefore maternally transmitted and deployment of *Wolbachia* in the field showed that it was efficiently passed from a female carrying *Wolbachia* to its progeny (Jiggins, 2017).



**Figure 16** Maternal transmission of *Wolbachia* in the *Wolbachia*-infected mosquitoes (Ross, 2018).

208. The male GM mosquitoes released into the environment could encounter and mate with WT female mosquitoes carrying *Wolbachia*. The resulting progeny (F1) would be all be males carrying *Wolbachia*, hemizygous for the tTAV gene.

209. In Figure 17 below, a schematic of the outcome of the F1 males subsequently mating with a WT female carrying *Wolbachia* or not, is included. If the subsequent cross involves a female not carrying *Wolbachia*, no offspring would be produced due to the cytoplasmic incompatibility described above.



**Figure 17 Schematic representation of the breeding of the GM male mosquito with wild type female mosquitoes with or without *Wolbachia* (drawn by OGTR).**

210. If the F1 male GM hemizygote mosquito mates with a WT female mosquito carrying *Wolbachia*, 50% of the offspring (25% male, 25% female) would not be GM and be free of the tTAV and DsRed2 genes and carry *Wolbachia* as per the local population. The other 50% of the progeny would be GM and carry the tTAV and DsRed2 genes. The female GM mosquitoes (25% of the progeny) would not reach adulthood. The male progeny (25% of the progeny) from this cross would survive and continue to subsequently transfer the tTAV and DsRed2 genes.

211. As discussed in Chapter 1, Section 4.2.10, *Wolbachia* can be established and function as intended in the parental wild type mosquitoes used to generate the GM mosquitoes. Therefore, it is highly likely that the inheritance and cytoplasmic incompatibility profile of *Wolbachia* will be maintained in the GM mosquitoes.

#### Reduced effectiveness of *Wolbachia* as a dengue control strategy

212. The maternal transmission of *Wolbachia* may not necessarily occur in 100% of the offspring. Regardless, a gradual reduction in overall mosquito population would occur through the reduction of the number of female mosquitoes due to the cytoplasmic incompatibility associated with *Wolbachia* transmission and the non-viability of GM female larva. If the release of GM mosquitoes is a one-off event, the tTAV and DsRed2 genes would rapidly be diluted in the population of mosquitoes and the number of WT female mosquitoes carrying *Wolbachia* or not would return in time to the numbers pre-release.

213. If the release of GM mosquitoes is a regular occurrence, the overall numbers of females would be progressively reduced but the percentage of females in the mosquito population carrying *Wolbachia* may increase. It is to be noted that the system described here does not consider the constant influx of WT *Ae. aegypti* from other sources or areas which also plays a role in the dynamics of those mosquito populations. If the release of the GM mosquitoes results in a sustained and

significant reduction, there may be an influx of mosquitoes free of *Wolbachia* from a neighbouring population, resulting in the local extinction of mosquitoes carrying *Wolbachia*. However, in those areas, it is still expected that fewer females would present, so it is highly unlikely that the transmission of arboviruses would increase.

214. As mentioned in Chapter 1 Section 1.2.1, there are various other methods in controlling the population of *Ae. aegypti* including the use of larvicides and insecticides or practices to reduce breeding areas. These methods already impact the mosquito population, including those mosquitoes carrying *Wolbachia*.

### **Potential harm**

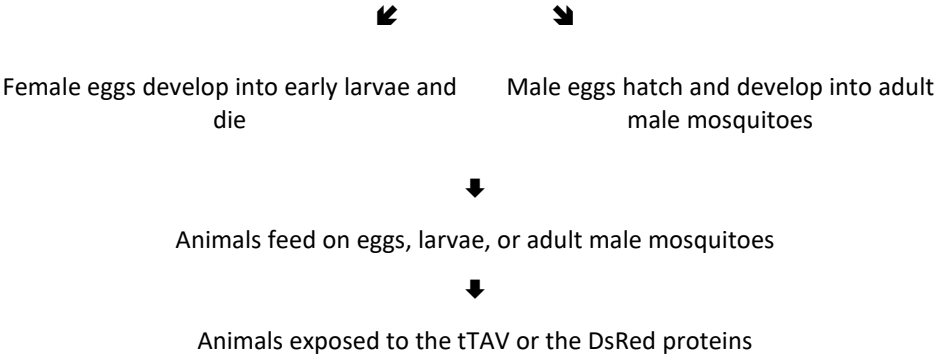
215. The release of the GM mosquitoes seeks a similar outcome to the release of mosquitoes containing *Wolbachia*, which is the prevention of dengue transmission. The continued release of the GM mosquitoes can suppress the female population but overall does not affect the transmission of *Wolbachia* in the *Ae. aegypti* population. Therefore, it is highly unlikely that the release of the GM mosquitoes would result in the increase of *Ae. aegypti* population and therefore in the increase of incidence of arbovirus outbreaks caused by *Ae. aegypti*.

216. The risk-benefit analysis of the deployment of the proposed GM mosquitoes and the comparison to already existing control methods, including *Wolbachia*-carrying *Ae. aegypti*, does not fall within the scope of the *Gene Technology Act 2000*, which aims to protect the health and safety of people and to protect the environment.

### **Conclusion**

217. The potential of the GM mosquitoes to increase the incidence of arbovirus outbreak is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

**2.4.4 Risk scenario 4**

Risk source	GM mosquitoes
<b>Causal pathway</b>	<p data-bbox="620 338 1171 367">Deployment of the rearing box in the environment</p> <div data-bbox="421 439 1362 792">  <pre> graph TD     A[Deployment of the rearing box in the environment] --&gt; B[Female eggs develop into early larvae and die]     A --&gt; C[Male eggs hatch and develop into adult male mosquitoes]     B --&gt; D[Animals feed on eggs, larvae, or adult male mosquitoes]     C --&gt; D     D --&gt; E[Animals exposed to the tTAV or the DsRed proteins]           </pre> </div>
<b>Potential harm</b>	Toxicity in animals

**Risk Source**

218. The source of potential harm for this postulated risk scenario is the GM eggs, larvae or adult mosquitoes.

**Causal Pathway**

219. Mosquito eggs, larvae and adults are a source of food for animals such as frogs, dragonflies or fish, some of which may be endangered native species. This risk scenario considers the potential toxicity associated with the ingestion of GM eggs, larvae and adult mosquitoes containing the tTAV and the DsRed proteins and its potential impact on animals in the environment.

220. Once the rearing boxes have been deployed, several source of food would be present in the environment for other animals to feed on including GM eggs and larvae and adult mosquitoes.

*Female eggs develop into larvae and die before adulthood*

221. Once the rearing boxes are deployed, GM embryos will develop into larvae. In GM female larvae, as the tTAV is expressed, it accumulates into the cells and the positive feedback loop is triggered. This positive feedback overwhelms the expression machinery of the cells, impeding the expression of other critical genes and resulting in the death of the larvae. tTAV proteins would remain within the larvae at a high concentration. The dead larvae would be located within the rearing boxes or could potentially fall from the rearing box and into the surrounding environment, and could be consumed by other insects, birds or other animals.

*Male eggs hatch and develop into adult male mosquitoes*

222. As tTAV levels in the eggs and the developing larvae are either extremely low or not present, the GM male larvae would develop into adult mosquitoes. It is expected that because the male mosquitoes have survived, the level of tTAV proteins in those GM adult mosquitoes would be very low. The applicant has provided western blot analysis demonstrating that only a small amount of DsRed and even a smaller amount of tTAV proteins are present in male homozygous mosquitoes and larvae (Section 4.2.6).

### DsRed2 expression

223. DsRed2 is expressed independently of the tTAV gene, and its expression is driven by the HR5-IE1 enhancer and promoter, derived from the *Autographa californica nuclear polyhedrosis virus* (AcNPV), an insect virus from the Baculoviridae family. It is expected that both GM female and male eggs, larvae and GM male adult mosquitoes would express this protein as it was introduced to allow for the identification of the construct.

224. The applicant has provided data in regard to the potential persistence of this protein in the environment and once ingested in the animal stomach. These data showed that those proteins are readily digested within 5 to 10 minutes (tTAV) and 1 to 5 mins (DsRed2) when exposed to the environmental proteases (proteinase K and subtilisin A) or simulated gastric fluid. The relatively short lifespan of these proteins when exposed to the environment or the digestive system minimises the likelihood of exposure to animals. The digestibility of novel proteins in SGF has also been correlated to reduced allergenicity as mentioned in Chapter 1, section 4.2.7 (Astwood et al., 1996; Herman et al., 2005).

### Exposure of Animals to tTAV and DsRed2 proteins

225. Numerous invertebrates have a diet consisting of mosquitoes and they include for example dragonflies (and their larvae), spiders, and ants. Other animals that feed on mosquitos include bats, turtles, and many birds. Some fish species have a specialised ability to consume mosquitoes and mosquito larvae. However, there are a limited number of species known to feed on *Ae. aegypti* as the mosquito habitat mainly consist of inside houses or outdoors in water-filled artificial containers in urban environments (OECD, 2018). As mentioned previously, much higher amounts of tTAV proteins would be found in GM female eggs or larvae. GM male mosquitoes are not expected to express high quantities of this protein.

226. As described in risk scenario 1, the applicant has provided data regarding the presence of signal peptides in the GM mosquitoes associated with these proteins, which could lead to the secretion of these proteins. Using bio-informatic tools, no signal peptides were detected. The applicant has also provided data regarding the glycosylation of the DsRed and the tTAV proteins. Glycosylation of proteins typically occurs in the endoplasmic reticulum and is important in facilitating the transport of proteins from the nucleus into the cytoplasm (He et al., 2024). It therefore provides an indication of the likelihood of a protein being secreted by a cell. Glycosylation was not detected above the limit of detection (50 pg per mosquito). This means that it is highly unlikely that these proteins are secreted by the mosquito cells so exposure to these proteins would require the cell to die and release its contents which would expose them to proteases that would quickly minimise the persistence of these proteins.

227. As previously mentioned in Chapter 1, section 4.2.7, the applicant has provided extensive bioinformatic data supporting the lack of toxicity of these two proteins.

228. In addition, DsRed2 has been used in other GMOs and its potential toxicity and allergenicity have been investigated in multiple context concluding that the DsRed protein was not toxic or allergenic when expressed in various organisms or when ingested by rodents (Dietrich and Maiss, 2002; Mikkelsen et al., 2003; Matsushima et al., 2010; Shemiakina et al., 2012; Lenard et al., 2016; Qureshi and Connolly, 2023).

229. The applicant has provided extensive toxicity data summarised in Table 4 in section 4.2.7. Those studies involve feeding vertebrates or invertebrates with GM mosquito larvae or other insect larvae modified to express DsRed and tTAV proteins. Toxicity was assessed in freshwater fish (guppy), Bobwhite quail, crayfish, elephant mosquitoes and carabid beetles. These studies involved monitoring for any increased mortality and any sublethal effects in organisms fed with a control WT insect larvae or a GM insect expressing DsRed or tTAV. Those studies did not identify any toxicity associated with the ingestion of the tTAV or the DsRed proteins.

230. The US EPA has conducted an additional study to assess potential toxicity in aquatic larvae in the area where a trial of a similar GM mosquito was conducted. No toxicity was detected in this study.

### Potential harm

231. Lethality in the female mosquito is caused by the disruption of other cellular processes due to the accumulation of the tTAV protein and not the protein itself. The positive feedback loop is responsible for the continuous expression of tTAV protein, thus disrupting the expression of other critical proteins for the mosquito larvae. In this scenario, exposure to the protein itself would not have the same effect as the expression cassette is not present, and therefore no positive feedback loop to drive its own expression is possible.

### Conclusion

232. The result of exposure of animals to proteins via the consumption of GM larvae or GM male mosquitoes leading to toxicity is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

#### 2.4.5 Risk scenario 5

Risk source	GM mosquitoes
Causal pathway	<p>Exposure of eggs/larvae to tetracycline present in environment</p> <p>OR</p> <p>Inactivation of the female self-limiting gene via inactivation of the OX5034 cassette</p> <p>↓</p> <p>GM female mosquitoes survive to adulthood.</p> <p>↓</p> <p>GM female mosquito bites an animal</p> <p>↓</p> <p>Exposure of the animal to DsRed and tTAV</p>
Potential harm	Toxicity in animals

### Risk Source

233. The source of potential harm for this postulated risk scenario is the GM mosquito.

### Causal Pathway

234. Since only female mosquitoes blood feed, it is highly unlikely that the introduction of GM male mosquitoes can result in the exposure animals of animals to tTAV and DsRed2. The presence tetracycline in the environment or a mutation in the OX5034 cassette could lead to the female mosquitoes surviving into adulthood. The surviving females could bite an animal in the environment resulting in toxicity in animal exposed to tTAV or DsRed via a mosquito bite.

#### Survival of GM female mosquitoes

235. As discussed in risk scenario 1, it is highly unlikely female mosquitoes can survive in the environment as:

- The female mosquito would either have to live in environment rich in tetracycline or have to undergo a mutation in the tTAV cassette, inactivating the expression of the tTAV protein,



- The applicant has provided data supporting the stability of the expression cassette across 49 generations,
- The applicant has provided data showing 100% penetrance of the female-specific self-limiting gene, meaning it is expected that no female would be released as part of the deployment of rearing boxes.

#### GM female mosquito bites an animal

236. *Ae. aegypti* almost exclusively feed on humans (75-99%) and at low frequencies (<1-19%) on other hosts (e.g. bovine, swine, cat, rat, and chicken) (Ponlawat and Harrington, 2005; Jansen et al., 2009). Exposure to the tTAV and the DsRed2 proteins in animals

237. If in the unlikely event that female mosquitoes survive to adulthood, the tTAV protein would be either not expressed at all or expressed at very low levels, not sufficient to result in the death of female larvae. A small amount of the tTAV protein or the DsRed protein may be present when the female GM mosquito bites an animal.

238. The applicant has provided data regarding the absence of signal peptides in the GM mosquitoes associated with these proteins. As mentioned in section 4.2.7 and in risk scenario 1, using bio-informatic tools, no signal peptides were detected. This means that it is highly unlikely that these proteins are secreted by the mosquito cells. Therefore, these proteins are unlikely to be found in the saliva of the mosquitoes and injected into the blood of a person via a bite.

239. If, however, a small quantity of either protein is transferred in the blood of a person, tTAV and DsRed proteins are not toxic (see risk scenario 1).

240. The applicant has provided data regarding the toxicity of ingested tTAV and DsRed2. Mice were orally fed extremely large amounts of protein (equivalent to about 18 million of mosquitoes were ingested for tTAV or 1.6 million mosquitoes were ingested for DsRed2). No toxicity was observed when a mouse was exposed to a large amount of these proteins. Given the concentration of proteins present in the gut and following digestion, due to the permeability of the gut-blood barrier, some whole or peptides derived from the digestion of DsRed2 or tTAV may end up crossing the blood barrier no toxicity was observed in these studies, which indicate that the presence of tTAV or DsRed2 in blood is unlikely to cause any adverse effect.

#### **Potential harm**

241. The potential harm has been described in risk scenario 4.

#### **Conclusion**

242. The result of exposure of animals to proteins via a mosquito bite leading to toxicity is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

### 2.4.6 Risk scenario 6

Risk source	GM male mosquitoes
Causal pathway	<p>Release of GM male mosquitoes</p> <p>↓</p> <p>Breeding with WT <i>Ae. aegypti</i></p> <p>OR</p> <p>Interbreeding with other <i>Aedes</i> species.</p> <p>↓</p> <p>Reduction in population of <i>Ae. aegypti</i> or other closely related species.</p> <p>↓</p> <p>Changes in dynamics of the population of <i>Ae. aegypti</i> or closely related mosquito species.</p>
Potential harm	Disturbance of the ecosystem

#### Causal Pathway

243. The primary purpose of the release of the GM mosquitoes is to reduce the population of *Ae. aegypti* to reduce dengue outbreaks. There may be indirect consequences if the GM mosquitoes interbreed with other species and reduce the population of other mosquito species. Mosquitoes such as *Ae. aegypti* can be a food source, play a role in the pollination of plants and, even as a recently introduced species, are part of a complex ecological balance. This risk scenario looks at the impact of the proposed release on the surrounding environment, including on populations of mosquito species but also the impact of this release on the dynamic of other populations of animals or plants reliant on the presence of *Ae. aegypti* in their ecological environment.

#### GM male mosquito mates with another species of mosquito

244. As described in Section 5.3, there are other mosquitoes that belong to the same subgenus (*Stegomyia*) in Australia (*Ae. aegypti queenslandensis*, *Ae. albopictus*, and *Ae. scutellaris*). The most common mosquito in Australia (*Ae. notoscriptus*) belongs to the same genus (*Aedes*) but a different subgenus (*Finlaya*) (Webb, 2016).

245. However, based on publicly available data described in Sections 5.3.2 to 5.3.4, it is highly unlikely that the GM mosquitoes (*Ae. aegypti*) could interbreed with other species of *Aedes* mosquitoes to produce viable offspring.

246. *Ae. albopictus* is another species of mosquito not endemic to Australia but native to Southeast Asia that has spread through many areas including the Pacific region. It has persisted in the Torres Strait and has now established a stable population in that region. It seems inevitable that this species establishes in Australia in similar niches occupied by *Ae. aegypti*. It is a vector for dengue and chikungunya. Studies have been conducted looking at the interbreeding between *Ae. aegypti* and *Ae. albopictus*. Mating between those two mosquito species was forced in a laboratory and as a result eggs were produced. However, those eggs never hatched to produce adult mosquitoes (Harper and Paulson, 1994). Interspecific matings can also occur in the wild between *Ae. aegypti* and *Ae. albopictus* but this is very rare and results in no viable progeny (Tripet et al., 2011).

247. Therefore, it is highly unlikely that the release of the GM mosquitoes could impact the population of the other species of mosquitoes that are present in Australia by passing the tTAV gene to their progeny.

GM male mosquito mates with wild type *Ae. aegypti*

248. The mating of GM male mosquitoes with wild type *Ae. aegypti* mosquitoes is the intent of the proposed deployment of the rearing boxes. A GM male mosquito would mate with a WT female mosquito. All female progeny would die, due to the expression of the tTAV gene in female larvae and the male F2 would transfer the tTAV gene to half of its offspring in subsequent cross. If the release is not repeated, stochastic modelling has predicted that the GM male mosquitoes carrying the transgene will disappear from the environment within 10 generations (Spinner et al., 2022). However, if there is regular release of GM male mosquitoes, it is expected that the tTAV cassette would spread into the local population of *Ae. aegypti* suppressing the population of mosquitoes. Therefore, in areas where the release occurred, *Ae. aegypti* population would progressively decrease.

Ecological niche left free for other mosquito species to occupy

249. The intent of the release is to suppress populations of *Ae. aegypti* which was shown to occur in trials of a similar GMO in Brazil (Carvalho et al., 2015). With the significant decrease of the *Ae. aegypti* population, the ecological niche previously occupied by this mosquito species is left free to be utilised by other species of mosquitoes. As mentioned in section 5.3, a number of other species are present in Australia, some are endemic, whilst some are only sporadically detected.

250. *Ae. notoscriptus* is common in Queensland and shares similarity with *Ae. aegypti* and inhabit similar niches to *Ae. aegypti* and hence it is possible that this mosquito population could occupy the vacated niche. However, this mosquito is already widely distributed in Australia (Section 5.3.4) and unlikely to have a negative ecological impact.

251. As mentioned in Section 5.3.3, *Ae. scutellaris* is a less well described mosquito that would share a similar niche to *Ae. aegypti*. Hence, it is possible that *Ae. scutellaris* could occupy any niches vacated if *Ae. aegypti* is eliminated from the population. This could potentially increase the population of *Ae. scutellaris*.

252. *Ae. albopictus*, although not endemic to Australia, appears to be the obvious mosquito species to occupy those ecological niches potentially left vacant by the release. This is because they share the same resources including laying and feeding grounds. In Singapore, a study was conducted in areas where male *Wolbachia*-infected *Ae. aegypti* were released to suppress wild population of *Ae. aegypti* (as progeny resulting from the cross between a male *Wolbachia*-infected mosquito and a WT female would not survive). The study followed the population of *Ae. albopictus* for two years and concluded that, while the population trend of *Ae. albopictus* varied across those two years, those populations did not increase in area where the release occurred (Wong et al., 2025).

253. The Brazilian National Technical Commission on Biosafety (CTNBio) has approved the commercial release of the GM mosquito (OX5034). In their technical report, they mentioned that monitoring of populations of *Ae. albopictus* was conducted between May 2015 and December 2018 by Oxitec while the release of GM mosquitoes occurred. *Ae. albopictus* was not detected in the ecological niches vacated by *Ae. aegypti* as a result of the release of the GM mosquitoes, although *Ae. albopictus* is present in the vicinity (National Technical Commission of Biosafety Brazil – note that the approval is in Portuguese). A similar study using another GM mosquito (OX513A) in West Panama also demonstrated that the sustained reduction (up to 93%) in *Ae. aegypti* through repeated release did not affect the population of *Ae. albopictus* (Gorman et al., 2016).

254. As mentioned in section 5.4, there are other species of mosquitoes that can carry diseases present in Australia. It is possible that these species could fill in the niches vacated by *Ae. aegypti*. However, they do not typically share similar habitats to *Ae. aegypti* and are predominantly found in

swamps or rural areas. Therefore, it is unlikely that the suppression of the population of *Ae. aegypti* translates into an increase of populations of these other mosquito species.

### Stronger fitness for hybrid mosquito

255. *Ae. aegypti* strains across the world, while extremely similar genotypically, are not identical (Santos et al., 2022). In the trials conducted in Brazil with the first-generation GM mosquito (OX513A), it was initially reported that the release resulted in the formation of a hybrid mosquito, a result from successive crosses between the release strain of *Ae. aegypti* and the target Jacobina strain in Brazil (Evans et al., 2019). However, there have been concerns expressed by some of the authors about the interpretation of the data involving the introgression and increased fitness of the hybrid mosquitoes (Evans et al., 2020).

256. In the report published by the US EPA in 2020 (US Environmental Protection Agency, 2020), hybrid fitness was assessed as unlikely both in terms of:

- vector competence, meaning that the resulting mosquito is not more likely to transmit an arbovirus, and
- fecundity and longevity for which the applicant has provided data showing that the longevity and fecundity is similar to the wild-type mosquito. Field trials have shown that their lifespan may be slightly lower than wild type population (Spinner et al., 2022).

257. The outcome of the proposed release is that the transgene would spread into the local population. However, it is highly unlikely that, as the result of the genetic modification, the mosquito would transmit arboviruses more efficiently or reproduce faster than the wild type mosquito as the transgene would not produce any viable female offspring. Other agencies would assess the vector competency profile in the original strain of *Ae. aegypti*.

258. Furthermore, and as mentioned in section 4.2.9, the GM mosquitoes are susceptible to the same insecticides used for the control of WT *Ae. aegypti*. They can therefore be controlled in a similar fashion than WT mosquitoes and do not have any advantage compared to WT population.

### Mosquitoes as a food source

259. As described in Section 3.1, there are natural predators of *Ae. aegypti* that feed on both aquatic (larvae and pupae) and adult stages of the mosquito. However, while most predators can eat mosquito larvae or adult mosquitoes, the predators are not commonly found in most *Ae. aegypti* habitats principally consisting of indoor (houses) or outdoor backyards (e.g. artificial containers) (OECD, 2018).

260. In Australia, the numbers/biomass of *Ae. aegypti* present in Cairns, Queensland, is small (an estimated 2g/ha) and it is highly unlikely that it would make a large contribution as a critical food source for any predators in Australia (OECD, 2018). It is anticipated that these predators would also feed on other mosquito strains and species, or other insects as there has been no indication that there are any species of animals/insects that solely rely on *Ae. aegypti* mosquitoes as their only food source.

261. Current practices for the control of dengue involves the use of insecticides to reduce the population of *Ae. aegypti* during and in between dengue outbreaks. *Ae. aegypti* numbers vary depending on temperature and rainfall (Duncombe et al., 2013; Reinhold et al., 2018; Rajarethinam et al., 2020). These natural or insecticide induced variations in the population would also cause fluctuations in food availability for predators which rely on *Ae. aegypti* as a food source.

### Mosquitoes as pollinators

262. *Ae. aegypti* and other mosquitoes especially males feed on sugar sources from plants and possibly serve as pollinators of some plant species, while females tend to be less dependent on plant sugar sources and rely on blood feeding.

263. While it is possible that *Ae. aegypti* could pollinate plants, it is highly unlikely that any plants are reliant solely on pollination by *Ae. aegypti*. An extensive review by Foster *et al* concluded that there is insufficient conclusive evidence that would suggest that, *Ae. aegypti* is an essential pollinator and that the elimination of the population would have conceivably negative ecological impact (Foster, 2024).

264. As mentioned in risk scenario 3, the population of mosquitoes such as *Ae. aegypti* varies over time with the use of insecticides, other control methods and ecological changes (e.g. wet/dry seasons). Those methods do not discriminate *Ae. aegypti* species but rather target all mosquito species. Suppression of mosquito populations via these more traditional control methods have not been reported to be associated with the decrease or extinction of other animals or plants.

### Potential harm

265. If the proposed release works as intended and regular release of the GM male mosquitoes occurs, it will result in a reduction of the local *Ae. aegypti* population. Data produced by the applicant from the trials authorised in Brazil and the USA provide an insight into the short-term impact of the release of the GM male mosquitoes. The applicant states that the impact of the release can be reversed if the deployment of the rearing boxes stops. This has been demonstrated in the short term after repeated and intensive deployment where the tTAV and the DsRed2 proteins can no longer be detected after 10 generations (roughly 3 months) (Spinner *et al.*, 2022). The decrease in *Ae. aegypti* populations may however result in another population of mosquitoes occupying the vacated niche or the reduction of other insect or vertebrates' population.

### Conclusion

266. The potential of the release of GM male mosquitoes to impact the balance of the ecosystem is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

## Section 3 Uncertainty

267. Uncertainty is an intrinsic part of risk analysis<sup>2</sup>. There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

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

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

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<sup>2</sup> 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.

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

270. For DIR-207 uncertainty is noted in relation to the long-term effect of this release on the local environment. If this strategy is adopted for the control of dengue, regular releases would be required for the sustained suppression of *Ae. aegypti*. Currently, insecticides are used to control this mosquito population and are often scaled up in the event of an outbreak. *Ae. aegypti* populations can therefore vary significantly over time. The long-term effect of a consistent suppression of *Ae. aegypti* population on the local ecosystem is unknown. Data from field trials in Brazil span over a period of roughly a year, and while the transgene itself is no longer detected, there is no available studies examining the effect of the release on populations of other mosquitoes. Another area of uncertainty is whether the data collected in Brazil and the United States are directly applicable to the Australian environment as no data were provided on the release of the GM mosquitoes in Australia.

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

## Section 4 Risk evaluation

272. 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.

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

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

274. Six risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether:

- the GM mosquito can persist in the environment;
- the exposure of people and animals to the proteins expressed in the GMO could lead to toxicity or allergenicity;
- the release of the GM mosquitoes can impact the numbers of mosquitoes or other animals in the environment; and
- the release of the GM mosquito can result in increased numbers of mosquitos that can transmit dengue.

275. 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.

276. In the context of the range of measures already in place, including the operating guidelines and requirements of the other regulatory agencies, and considering both the short and long term, none of these scenarios was identified as representing a substantive risk requiring further assessment. The principal reasons for this include:

- the population of the GMO is unlikely to persist indefinitely in the environment;

- the proteins expressed in the GMO are not toxic or allergenic;
- the release of only male mosquitoes and the death of female offspring would not increase the number of mosquitoes that can transmit arboviruses;
- *Ae. aegypti* is not a main food source or pollinator.

277. Therefore, any risks to the health and safety of people, or the environment, from the proposed commercial supply of the GMO 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.

278. Control measures may be imposed by the APVMA. However, since an application is yet to be submitted with the APVMA, additional measures to maintain elements of the risk context, including ongoing oversight are considered in Chapter 3.

## Chapter 3 Risk management plan

### Section 1 Background

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

280. 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.

281. 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.

282. 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

283. The risk assessment of risk scenarios listed in Chapter 2, concluded that there are negligible risks to people and the environment from the proposed release of the GM mosquitoes in the context of the scale of the proposed release and the receiving environment. The risk evaluation concluded that no control measures are required to treat these negligible risks.

### Section 3 General risk management

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

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

#### 3.1 Applicant suitability

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

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



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

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

### **3.2 Testing methodology**

287. If a licence were issued, Oxitec Australia have already provided a method to the Regulator for the reliable detection of the GM mosquito, and the presence of the introduced genetic materials in a recipient organism. The methods consist of visual detection of DsRed protein in larvae and a PCR test to detect DsRed and tTAV genes.

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

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

### **3.4 Reporting requirements**

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

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

290. Unintended effects of the release include any unintended environment impacts and if the GMO is not behaving as intended.

291. This condition applies for the duration of the licence and allows the Regulator to have ongoing oversight of this release by being made aware of any changes to the risk context in which this application was evaluated. This would include any unintended short or long terms harm from the release of the GM mosquitoes.

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

293. 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).

294. If issued, the licence would also require the licence holder to notify the Regulator of the authorisations by the APVMA, DAFF, DCCEE and any measure imposed by the Queensland Government.

295. All the data provided as part of this application have been gathered in other parts of the world, either in Brazil or the USA. As mentioned in section 3, uncertainties remain whether the data provided as part of this application is entirely applicable to the release in the Australian environment.

### **3.5 Monitoring for compliance**

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

297. 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**

298. 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.

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

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

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

### **4.1 Adverse effects reporting system**

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

### **4.2 Requirement to monitor specific indicators of harm**

301. 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.

302. 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.

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

304. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. No specific indicators of harm have been identified in this RARMP for application DIR 207. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

305. 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**

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

## **Section 5 Conclusions of the consultation RARMP**

307. The risk assessment concludes that the release of this GM mosquito poses negligible risks to the health and safety of people and the environment as a result of gene technology.

308. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, if a licence were to be issued, general conditions were also included in the draft licence to ensure that there is ongoing oversight of the GM mosquito.

## References

- Agriculture Victoria (2024). Vets dispensing Oxytetracycline hydrochloride to beekeepers for European foulbrood. Accessed: 17 February 2025.
- Akhter, S., Bhat, M.A., Ahmed, S., and Siddiqui, W.A. (2024). Antibiotic residue contamination in the aquatic environment, sources and associated potential health risks. *Environ Geochem Health* 46, 387.
- Alieva, N.O., Konzen, K.A., Field, S.F., Meleshkevitch, E.A., Hunt, M.E., Beltran-Ramirez, V., Miller, D.J., *et al.* (2008). Diversity and evolution of coral fluorescent proteins. *PLoS One* 3, e2680.
- Andrianjakarivony, H.F., Damiens, D., Marquereau, L., Gaudillat, B., Habchi-Hanriot, N., and Gouagna, L.C. (2022). Could Sterile *Aedes albopictus* Male Releases Interfere with *Aedes aegypti* Population in Reunion Island? *Insects* 13.
- Astwood, J.D., Leach, J.N., and Fuchs, R.L. (1996). Stability of food allergens to digestion in vitro. *Nature Biotechnology* 14, 1269-1273.
- Bammer, G., and Smithson, M. (2008). *Uncertainty and risk: Multidisciplinary perspectives* (London: Earthscan).
- Bargielowski, I., Honório, N.A., Blosser, E.M., and Lounibos, L.P. (2019). Rapid Loss of Resistance to Satyrization in Invasive Mosquitoes and the Effects of Age on Interspecific Mating Frequency. *Journal of Medical Entomology* 56, 329-333.
- Bargielowski, I.E., Lounibos, L.P., Shin, D., Smartt, C.T., Carrasquilla, M.C., Henry, A., Navarro, J.C., *et al.* (2015). Widespread evidence for interspecific mating between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in nature. *Infect Genet Evol* 36, 456-461.
- Battaglia, V., Agostini, V., Moroni, E., Colombo, G., Lombardo, G., Rambaldi Migliore, N., Gabrieli, P., *et al.* (2022). The worldwide spread of *Aedes albopictus*: New insights from mitogenomes. *Front Genet* 13, 931163.
- Beebe, N.W., Pagendam, D., Trewin, B.J., Boomer, A., Bradford, M., Ford, A., Liddington, C., *et al.* (2021). Releasing incompatible males drives strong suppression across populations of wild and Wolbachia-carrying *Aedes aegypti* in Australia. *Proc Natl Acad Sci U S A* 118.
- Bhattacharyya, J., and Roelke, D.L. (2024). Wolbachia-based mosquito control: Environmental perspectives on population suppression and replacement strategies. *Acta Trop* 262, 107517.
- Bimbile Somda, N.S., Maiga, H., Mamai, W., Bakhoun, T., Wallner, T., Poda, S.B., Yamada, H., *et al.* (2022). Adult mosquito predation and potential impact on the sterile insect technique. *Sci Rep* 12, 2561.
- Bondad-Reantaso, M.G., MacKinnon, B., Karunasagar, I., Fridman, S., Alday-Sanz, V., Brun, E., Le Groumellec, M., *et al.* (2023). Review of alternatives to antibiotic use in aquaculture. *Reviews in Aquaculture* 15, 1421-1451.
- Cane, R.P.L., D.; Turbitt, E.; Chambers, G.K. (2020). Molecular phylogenetic analysis of New Zealand mosquito species. *New Zealand Journal of Zoology* 47, 324-349.
- Canyon, D., and Hii, J. (1999). Insecticide susceptibility status of *Aedes aegypti* (Diptera: Culicidae) from Townsville. *Australian Journal of Entomology* 38, 40-43.

- Carvalho, E., David, G.S., and Silva, R.J. (2012). Health and Environment in Aquaculture (IntechOpen).
- Chihota, C.M., Rennie, L.F., Kitching, R.P., and Mellor, P.S. (2001). Mechanical transmission of lumpy skin disease virus by *Aedes aegypti* (Diptera: Culicidae). *Epidemiol Infect* 126, 317-321.
- Chopra, I., and Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* 65, 232-260 ; second page, table of contents.
- Clark, A.J., and Brinkley, T. (2001). Risk management: for climate, agriculture and policy. (Canberra: Commonwealth of Australia).
- Clemons, A., Mori, A., Haugen, M., Severson, D.W., and Duman-Scheel, M. (2010). Culturing and egg collection of *Aedes aegypti*. *Cold Spring Harb Protoc* 2010, pdb prot5507.
- Codex Alimentarius Commission (2003). Guideline for the conduct of food safety assessment of foods derived from the recombinant-DNA plants CAC/GL 45-2003.
- Codex Alimentarius Commission (2008). Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA Microorganisms. CAC/GL 68-2008 CAC/GL 46-:1–17.
- Codex Alimentarius Commission & WHO (2009). Foods derived from modern biotechnology.
- Communicable Diseases Network Australia (2015). CDNA National Guidelines for Public Health Units, D.o. Health, ed.
- CSIRO (2024). About the *Aedes aegypti* mosquito. (CSIRO) Accessed: 7 November 2024.
- Department of Agriculture Fisheries and Forestry (2023a). Australia's freedom from lumpy skin disease, Department of Agriculture Fisheries and Forestry, ed. (Australian Government).
- Department of Agriculture Fisheries and Forestry (2023b). Sterile Insect Technique use in Australia. Accessed: 21 February 2025.
- Department of Health and Aged Care (2025). National Notifiable Disease Surveillance System (NNDSS). Accessed: 13 January 2025.
- Dietrich, C., and Maiss, E. (2002). Red Fluorescent Protein DsRed from *Discosoma* sp. as a Reporter Protein in Higher Plants. *BioTechniques* 32, 286-291.
- Duncombe, J., Clements, A., Davis, J., Hu, W., Weinstein, P., and Ritchie, S. (2013). Spatiotemporal patterns of *Aedes aegypti* populations in Cairns, Australia: assessing drivers of dengue transmission. *Trop Med Int Health* 18, 839-849.
- Endersby-Harshman, N.M., Wuliandari, J.R., Harshman, L.G., Frohn, V., Johnson, B.J., Ritchie, S.A., and Hoffmann, A.A. (2017). Pyrethroid Susceptibility Has Been Maintained in the Dengue Vector, *Aedes aegypti* (Diptera: Culicidae), in Queensland, Australia. *Journal of Medical Entomology* 54, 1649-1658.
- Evans, B.R., Kotsakiozi, P., Costa-da-Silva, A.L., Ioshino, R.S., Garziera, L., Pedrosa, M.C., Malavasi, A., *et al.* (2019). Transgenic *Aedes aegypti* Mosquitoes Transfer Genes into a Natural Population. *Scientific Reports* 9, 13047.

- Evans, B.R., Kotsakiozi, P., Costa-da-Silva, A.L., Ioshino, R.S., Garziera, L., Pedrosa, M.C., Malavasi, A., *et al.* (2020). Editorial Expression of Concern: Transgenic *Aedes aegypti* Mosquitoes Transfer Genes into a Natural Population. *Scientific Reports* 10, 5524.
- Ferreira, Q.R., Lemos, F.F.B., Moura, M.N., Nascimento, J.O.S., Novaes, A.F., Barcelos, I.S., Fernandes, L.A., *et al.* (2023). Role of the Microbiome in *Aedes* spp. Vector Competence: What Do We Know? *Viruses* 15.
- Flores, H.A., and O'Neill, S.L. (2018). Controlling vector-borne diseases by releasing modified mosquitoes. *Nat Rev Microbiol* 16, 508-518.
- Foster, W.A. (2024). Mosquito pollination of plants: an overview of their role and an assessment of the possible contribution of disease vectors. *Transgenic Res* 33, 297-322.
- Frost, E. (2021). European foulbrood and its control, N.D.o.P. Industries, ed.
- Gallia, G.L., and Khalili, K. (1998). Evaluation of an autoregulatory tetracycline regulated system. *Oncogene* 16, 1879-1884.
- Gloria-Soria, A., Ayala, D., Bheecarry, A., Calderon-Arguedas, O., Chadee, D.D., Chiappero, M., Coetzee, M., *et al.* (2016). Global genetic diversity of *Aedes aegypti*. *Mol Ecol* 25, 5377-5395.
- Gloria-Soria, A., Soghigian, J., Kellner, D., and Powell, J.R. (2019). Genetic diversity of laboratory strains and implications for research: The case of *Aedes aegypti*. *PLoS Negl Trop Dis* 13, e0007930.
- Gorman, K., Young, J., Pineda, L., Márquez, R., Sosa, N., Bernal, D., Torres, R., *et al.* (2016). Short-term suppression of *Aedes aegypti* using genetic control does not facilitate *Aedes albopictus*. *Pest Manag Sci* 72, 618-628.
- Gossen, M., and Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A* 89, 5547-5551.
- Gunara, N.P., Joelianto, E., and Ahmad, I. (2023). Identification of *Aedes aegypti* and *Aedes albopictus* eggs based on image processing and elliptic fourier analysis. *Sci Rep* 13, 17395.
- Haga, J., Enosawa, S., and Kobayashi, E. (2017). Cell Therapy for Liver Disease Using Bioimaging Rats. *Cell Med* 9, 3-7.
- Harper, J.P., and Paulson, S.L. (1994). Reproductive isolation between Florida strains of *Aedes aegypti* and *Aedes albopictus*. *J Am Mosq Control Assoc* 10, 88-92.
- Hayes, K.R. (2004). Ecological implications of GMOs: robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for genetically modified organisms. (Tasmania: CSIRO Division of Marine Research).
- He, M., Zhou, X., and Wang, X. (2024). Glycosylation: mechanisms, biological functions and clinical implications. *Signal Transduction and Targeted Therapy* 9, 194.
- Herman, R.A., Korjagin, V.A., and Schafer, B.W. (2005). Quantitative measurement of protein digestion in simulated gastric fluid. *Regulatory Toxicology and Pharmacology* 41, 175-184.

- Hoffmann, A.A., Iturbe-Ormaetxe, I., Callahan, A.G., Phillips, B.L., Billington, K., Axford, J.K., Montgomery, B., *et al.* (2014). Stability of the wMel Wolbachia Infection following invasion into *Aedes aegypti* populations. *PLoS Negl Trop Dis* 8, e3115.
- Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., Greenfield, M., *et al.* (2011). Successful establishment of Wolbachia in *Aedes* populations to suppress dengue transmission. *Nature* 476, 454-457.
- Hu, G.-Y., Ma, J.-Y., Li, F., Zhao, J.-R., Xu, F.-C., Yang, W.-W., Yuan, M., *et al.* (2022). Optimizing the Protein Fluorescence Reporting System for Somatic Embryogenesis Regeneration Screening and Visual Labeling of Functional Genes in Cotton. *Frontiers in Plant Science* 12.
- Huai, D., Wu, J., Xue, X., Hu, M., Zhi, C., Pandey, M.K., Liu, N., *et al.* (2023). Red fluorescence protein (DsRed2) promotes the screening efficiency in peanut genetic transformation. *Frontiers in Plant Science* 14.
- Huang, Y.S., Higgs, S., and Vanlandingham, D.L. (2019). Arbovirus-Mosquito Vector-Host Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses. *Front Microbiol* 10, 22.
- Jach, G., Binot, E., Frings, S., Luxa, K., and Schell, J. (2001). Use of red fluorescent protein from *Discosoma* sp. (dsRED) as a reporter for plant gene expression. *Plant J* 28, 483-491.
- James, C., Harfouche, M., Welton, N.J., Turner, K.M., Abu-Raddad, L.J., Gottlieb, S.L., and Looker, K.J. (2020). Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bull World Health Organ* 98, 315-329.
- Jansen, C.C., Webb, C.E., Graham, G.C., Craig, S.B., Zborowski, P., Ritchie, S.A., Russell, R.C., *et al.* (2009). Blood sources of mosquitoes collected from urban and peri-urban environments in eastern Australia with species-specific molecular analysis of avian blood meals. *Am J Trop Med Hyg* 81, 849-857.
- Jasinskiene, N., Coates, C.J., Benedict, M.Q., Cornel, A.J., Rafferty, C.S., James, A.A., and Collins, F.H. (1998). Stable transformation of the yellow fever mosquito, *Aedes aegypti*, with the Hermes element from the housefly. *Proc Natl Acad Sci U S A* 95, 3743-3747.
- Jiggins, F.M. (2017). The spread of Wolbachia through mosquito populations. *PLoS Biol* 15, e2002780.
- Kaur, R., Shropshire, J.D., Cross, K.L., Leigh, B., Mansueto, A.J., Stewart, V., Bordenstein, S.R., *et al.* (2021). Living in the endosymbiotic world of Wolbachia: A centennial review. *Cell Host Microbe* 29, 879-893.
- Knope, K., Doggett, S.L., Jansen, C.C., Johansen, C.A., Kurucz, N., Feldman, R., Lynch, S.E., *et al.* (2019). Arboviral diseases and malaria in Australia, 2014-15: Annual report of the National Arbovirus and Malaria Advisory Committee. *Commun Dis Intell* (2018) 43.
- Langham, F., and Cheng, A.C. (2019). Antibiotic use in animals and humans in Australia. *Med J Aust* 211, 159-160 e151.
- Lee, H.L., Aramu, M., Nazni, W.A., Selvi, S., and Vasan, S. (2009). No evidence for successful interspecific cross-mating of transgenic *Aedes aegypti* (L.) and wild type *Aedes albopictus* Skuse. *Trop Biomed* 26, 312-319.

- Lenard, A., Alghisi, E., Daff, H., Donzelli, M., McGinnis, C., and Lengerke, C. (2016). Using zebrafish to model erythroid lineage toxicity and regeneration. *Haematologica* 101, e164-167.
- Li, C., Bei, T., Niu, Z., Guo, X., Wang, M., Lu, H., Gu, X., *et al.* (2019). Adhesion and Colonization of the Probiotic *Lactobacillus rhamnosus* Labeled by Dsred2 in Mouse Gut. *Curr Microbiol* 76, 896-903.
- Li, J., O'Brien, J.W., Tscharke, B.J., He, C., Shimko, K.M., Shao, X., Zhai, N., *et al.* (2024). National survey of the occurrence of antimicrobial agents in Australian wastewater and their socioeconomic correlates. *Nature Water* 2, 1166-1177.
- Mack, A. (2016). Global Health Impacts of Vector-Borne Diseases: Workshop Summary. In *The National Academies Press, E. National Academies of Sciences, and Medicine, ed. (Washington, DC).*
- Mann, D.G., Abercrombie, L.L., Rudis, M.R., Millwood, R.J., Dunlap, J.R., and Stewart, C.N., Jr. (2012). Very bright orange fluorescent plants: endoplasmic reticulum targeting of orange fluorescent proteins as visual reporters in transgenic plants. *BMC Biotechnol* 12, 17.
- Maruyama, M., Nishio, T., Yoshida, T., Ishida, C., Ishida, K., Watanabe, Y., Nishikawa, M., *et al.* (2004). Simultaneous detection of DsRed2-tagged and EGFP-tagged human beta-interferons in the same single cells. *J Cell Biochem* 93, 497-502.
- Massonnet-Bruneel, B., Corre-Catelin, N., Lacroix, R., Lees, R.S., Hoang, K.P., Nimmo, D., Alphey, L., *et al.* (2013). Fitness of transgenic mosquito *Aedes aegypti* males carrying a dominant lethal genetic system. *PLoS One* 8, e62711.
- Masters, S.W., Knappek, K.J., and Kendall, L.V. (2020). Rearing *Aedes aegypti* Mosquitoes in a laboratory setting. *Lab Animal Sci Prof* 55, 42-45.
- Matsushima, H., Ogawa, Y., Miyazaki, T., Tanaka, H., Nishibu, A., and Takashima, A. (2010). Intravital Imaging of IL-1 $\beta$  Production in Skin. *Journal of Investigative Dermatology* 130, 1571-1580.
- Matz, M.V., Fradkov, A.F., Labas, Y.A., Savitsky, A.P., Zarskiy, A.G., Markelov, M.L., and Lukyanov, S.A. (1999). Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat Biotechnol* 17, 969-973.
- Meier, C.J., Rouhier, M.F., and Hillyer, J.F. (2022). Chemical Control of Mosquitoes and the Pesticide Treadmill: A Case for Photosensitive Insecticides as Larvicides. *Insects* 13.
- Mikkelsen, L., Sarrocco, S., Lübeck, M., and Jensen, D.F. (2003). Expression of the red fluorescent protein DsRed-Express in filamentous ascomycete fungi. *FEMS Microbiology Letters* 223, 135-139.
- Moore, M., Sylla, M., Goss, L., Burugu, M.W., Sang, R., Kamau, L.W., Kenya, E.U., *et al.* (2013). Dual African origins of global *Aedes aegypti* s.l. populations revealed by mitochondrial DNA. *PLoS Negl Trop Dis* 7, e2175.
- Moore, P.R., Johnson, P.H., Smith, G.A., Ritchie, S.A., and Van Den Hurk, A.F. (2007). Infection and dissemination of dengue virus type 2 in *Aedes aegypti*, *Aedes albopictus*, and *Aedes scutellaris* from the Torres Strait, Australia. *J Am Mosq Control Assoc* 23, 383-388.
- Muir, L.E., and Kay, B.H. (1998). *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in northern Australia. *Am J Trop Med Hyg* 58, 277-282.



- Munoz, I., Carrillo, M., Zanuy, S., and Gomez, A. (2005). Regulation of exogenous gene expression in fish cells: an evaluation of different versions of the tetracycline-regulated system. *Gene* 363, 173-182.
- Murakami, T., and Kobayashi, E. (2005). Color-engineered rats and luminescent LacZ imaging: a new platform to visualize biological processes. *Journal of Biomedical Optics* 10, 041204.
- Muzari, M.O., Davis, J., Bellwood, R., Crunkhorn, B., Gunn, E., Sabatino, U., and Gair, R. (2019). Dominance of the tiger: The displacement of *Aedes aegypti* by *Aedes albopictus* in parts of the Torres Strait, Australia. *Commun Dis Intell* (2018) 43.
- Nahalkova, J., and Fatehi, J. (2003). Red fluorescent protein (DsRed2) as a novel reporter in *Fusarium oxysporum* f. sp. *lycopersici*. *FEMS Microbiology Letters* 225, 305-309.
- Naidoo, J., and Young, D. (2012). Gene regulation systems for gene therapy applications in the central nervous system. *Neurol Res Int* 2012, 595410.
- Naidoo, K., and Oliver, S.V. (2024). Gene drives: an alternative approach to malaria control? *Gene Ther.*
- National Arbovirus and Malaria Advisory Committee (2015). Framework for the surveillance, prevention and control of dengue virus infection in Australia, D.o. Health, ed.
- Nishizawa, K., Kita, Y., Kitayama, M., and Ishimoto, M. (2006). A red fluorescent protein, DsRed2, as a visual reporter for transient expression and stable transformation in soybean. *Plant Cell Rep* 25, 1355-1361.
- Nordin, O., Donald, W., Ming, W.H., Ney, T.G., Mohamed, K.A., Halim, N.A., Winskill, P., *et al.* (2013). Oral ingestion of transgenic RIDL *Ae. aegypti* larvae has no negative effect on two predator *Toxorhynchites* species. *PLoS One* 8, e58805.
- O'Brochta, D.A., Alford, R.T., Pilitt, K.L., Aluvihare, C.U., and Harrell, R.A., 2nd (2011). piggyBac transposon remobilization and enhancer detection in *Anopheles* mosquitoes. *Proc Natl Acad Sci U S A* 108, 16339-16344.
- O'Neill, S.L., Ryan, P.A., Turley, A.P., Wilson, G., Retzki, K., Iturbe-Ormaetxe, I., Dong, Y., *et al.* (2018). Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. *Gates Open Res* 2, 36.
- OECD (2002). Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing.
- OECD (2018). Consensus document on the biology of mosquito *Aedes aegypti*. Report No. 65. (OECD).
- OECD (2019). Test No. 203: Fish, Acute Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing.
- OGTR (2013). Risk Analysis Framework 2013. (Office of the Gene Technology Regulator).
- Ogunlade, S.T., Adekunle, A.I., Meehan, M.T., and McBryde, E.S. (2023). Quantifying the impact of *Wolbachia* releases on dengue infection in Townsville, Australia. *Sci Rep* 13, 14932.

- Ogunlade, S.T., Meehan, M.T., Adekunle, A.I., Rojas, D.P., Adegboye, O.A., and McBryde, E.S. (2021). A Review: Aedes-Borne Arboviral Infections, Controls and Wolbachia-Based Strategies. *Vaccines* (Basel) 9.
- Olson, M.F., Garcia-Luna, S., Juarez, J.G., Martin, E., Harrington, L.C., Eubanks, M.D., Badillo-Vargas, I.E., *et al.* (2020). Sugar Feeding Patterns for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) Mosquitoes in South Texas. *J Med Entomol* 57, 1111-1119.
- Palavesam, A., Esnault, C., and O'Brochta, D.A. (2013). Post-integration silencing of piggyBac transposable elements in *Aedes aegypti*. *PLoS One* 8, e68454.
- Paris, V., Rane, R.V., Mee, P.T., Lynch, S.E., Hoffmann, A.A., and Schmidt, T.L. (2023). Urban population structure and dispersal of an Australian mosquito (*Aedes notoscriptus*) involved in disease transmission. *Heredity* (Edinb) 130, 99-108.
- Ponlawat, A., and Harrington, L.C. (2005). Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *J Med Entomol* 42, 844-849.
- Powell, J.R., and Tabachnick, W.J. (2013). History of domestication and spread of *Aedes aegypti*--a review. *Mem Inst Oswaldo Cruz* 108 Suppl 1, 11-17.
- Pray, L. (2008). Transposons: The jumping genes. *Nature Education* 1(1), 204.
- Pyke, A.T., Shivas, M.A., Darbro, J.M., Onn, M.B., Johnson, P.H., Crunkhorn, A., Montgomery, I., *et al.* (2021). Uncovering the genetic diversity within the *Aedes notoscriptus* virome and isolation of new viruses from this highly urbanised and invasive mosquito. *Virus Evol* 7, veab082.
- Queensland Government (2017). Treatment advice for Pest Management Technicians – For the control of *Aedes aegypti* mosquito (vector of Zika and dengue), Q. Health, ed.
- Queensland Government (2021). Dengue: virus, fever and mosquitoes. Accessed: 7 November 2024.
- Queensland Health (2015). Queensland dengue management plan 2015–2020.
- Qureshi, A., and Connolly, J.B. (2023). Bioinformatic and literature assessment of toxicity and allergenicity of a CRISPR-Cas9 engineered gene drive to control *Anopheles gambiae* the mosquito vector of human malaria. *Malar J* 22, 234.
- Rahul, A., Reegan, A.D., Shriram, A.N., Fouque, F., and Rahi, M. (2024). Innovative sterile male release strategies for *Aedes* mosquito control: progress and challenges in integrating evidence of mosquito population suppression with epidemiological impact. *Infectious Diseases of Poverty* 13, 91.
- Rajarethinam, J., Ong, J., Neo, Z.W., Ng, L.C., and Aik, J. (2020). Distribution and seasonal fluctuations of *Ae. aegypti* and *Ae. albopictus* larval and pupae in residential areas in an urban landscape. *PLoS Negl Trop Dis* 14, e0008209.
- Rakotonirina, A., Dauga, C., Pol, M., Hide, M., Vuth, L., Ballan, V., Kilama, S., *et al.* (2024). Speciation patterns of *Aedes* mosquitoes in the Scutellaris Group: a mitochondrial perspective. *Scientific Reports* 14, 10930.
- Raquin, V., and Lambrechts, L. (2017). Dengue virus replicates and accumulates in *Aedes aegypti* salivary glands. *Virology* 507, 75-81.

Rasic, G., Filipovic, I., Callahan, A.G., Stanford, D., Chan, A., Lam-Phua, S.G., Tan, C.H., *et al.* (2016). The queenslandensis and the type Form of the Dengue Fever Mosquito (*Aedes aegypti* L.) Are Genomically Indistinguishable. *PLoS Negl Trop Dis* 10, e0005096.

Reinhold, J.M., Lazzari, C.R., and Lahondère, C. (2018). Effects of the Environmental Temperature on *Aedes aegypti* and *Aedes albopictus* Mosquitoes: A Review. *Insects* 9.

Riana, E., Sri-In, C., Songkasupa, T., Bartholomay, L.C., Thontiravong, A., and Tiawsirisup, S. (2024). Infection, dissemination, and transmission of lumpy skin disease virus in *Aedes aegypti* (Linnaeus), *Culex tritaeniorhynchus* (Giles), and *Culex quinquefasciatus* (Say) mosquitoes. *Acta Tropica* 254, 107205.

Ross, P. (2018). WOLBACHIA BACTERIA IN ACTION | How we're using naturally occurring bacteria to stop mosquitoes from spreading disease. (Pest & Environmental Adaptation Research Group (PEAR), University of Melbourne) Accessed: 17 February 2025.

Russell, R.C., Webb, C.E., Williams, C.R., and Ritchie, S.A. (2005). Mark-release-recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. *Med Vet Entomol* 19, 451-457.

Ryan, P.A., Turley, A.P., Wilson, G., Hurst, T.P., Retzki, K., Brown-Kenyon, J., Hodgson, L., *et al.* (2019). Establishment of wMel Wolbachia in *Aedes aegypti* mosquitoes and reduction of local dengue transmission in Cairns and surrounding locations in northern Queensland, Australia. *Gates Open Res* 3, 1547.

Saingamsook, J., Saeung, A., Yanola, J., Lumjuan, N., Walton, C., and Somboon, P. (2017). A multiplex PCR for detection of knockdown resistance mutations, V1016G and F1534C, in pyrethroid-resistant *Aedes aegypti*. *Parasit Vectors* 10, 465.

Sanchez-Aldana-Sanchez, G.A., Liedo, P., Bond, J.G., and Dor, A. (2023). Release of sterile *Aedes aegypti* mosquitoes: chilling effect on mass-reared males survival and escape ability and on irradiated males sexual competitiveness. *Sci Rep* 13, 3797.

Santos, G.V.d., Arduino, M.d.B., and Serpa, L.L.N. (2022). *Aedes aegypti queenslandensis*: first geographic occurrence in Brazil and epidemiological implications. *Revista Brasileira de Entomologia* 66.

Schmidt, T.L., Barton, N.H., Rasic, G., Turley, A.P., Montgomery, B.L., Iturbe-Ormaetxe, I., Cook, P.E., *et al.* (2017). Local introduction and heterogeneous spatial spread of dengue-suppressing Wolbachia through an urban population of *Aedes aegypti*. *PLoS Biol* 15, e2001894.

Schmidt, T.L., Endersby-Harshman, N.M., van Rooyen, A.R.J., Katusele, M., Vinit, R., Robinson, L.J., Laman, M., *et al.* (2024). Global, asynchronous partial sweeps at multiple insecticide resistance genes in *Aedes* mosquitoes. *Nat Commun* 15, 6251.

Schönig, K., Freundlieb, S., and Gossen, M. (2013). Tet-Transgenic Rodents: a comprehensive, up-to date database. *Transgenic Research* 22, 251-254.

Scitable (2014). Dengue Transmission. (Nature Education) Accessed: 17 February 2025.

Scolari, F., Casiraghi, M., and Bonizzoni, M. (2019). *Aedes* spp. and Their Microbiota: A Review. *Frontiers in Microbiology* 10.

- Sethuraman, N., Fraser, M.J., Jr., Eggleston, P., and O'Brochta, D.A. (2007). Post-integration stability of piggyBac in *Aedes aegypti*. *Insect Biochem Mol Biol* 37, 941-951.
- Shemiakina, I.I., Ermakova, G.V., Cranfill, P.J., Baird, M.A., Evans, R.A., Souslova, E.A., Staroverov, D.B., *et al.* (2012). A monomeric red fluorescent protein with low cytotoxicity. *Nat Commun* 3, 1204.
- Shutter, M.C., and Akhondi, H. (2025). Tetracycline. In StatPearls (Treasure Island (FL) ineligible companies. Disclosure: Hossein Akhondi declares no relevant financial relationships with ineligible companies.).
- Skelton, E., Rances, E., Frentiu, F.D., Kusmintarsih, E.S., Iturbe-Ormaetxe, I., Caragata, E.P., Woolfit, M., *et al.* (2016). A Native *Wolbachia* Endosymbiont Does Not Limit Dengue Virus Infection in the Mosquito *Aedes notoscriptus* (Diptera: Culicidae). *J Med Entomol* 53, 401-408.
- Souza-Neto, J.A., Powell, J.R., and Bonizzoni, M. (2019). *Aedes aegypti* vector competence studies: A review. *Infection, Genetics and Evolution* 67, 191-209.
- Spinner, S.A.M., Barnes, Z.H., Puinean, A.M., Gray, P., Dafa'alla, T., Phillips, C.E., Nascimento de Souza, C., *et al.* (2022). New self-sexing *Aedes aegypti* strain eliminates barriers to scalable and sustainable vector control for governments and communities in dengue-prone environments. *Front Bioeng Biotechnol* 10, 975786.
- Stieger, K., Belbellaa, B., Le Guiner, C., Moullier, P., and Rolling, F. (2009). In vivo gene regulation using tetracycline-regulatable systems. *Adv Drug Deliv Rev* 61, 527-541.
- Teufel, F., Almagro Armenteros, J.J., Johansen, A.R., Gíslason, M.H., Pihl, S.I., Tsirigos, K.D., Winther, O., *et al.* (2022). SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nature Biotechnology* 40, 1023-1025.
- Thumuluri, V., Almagro Armenteros, J.J., Johansen, A.R., Nielsen, H., and Winther, O. (2022). DeepLoc 2.0: multi-label subcellular localization prediction using protein language models. *Nucleic Acids Res* 50, W228-w234.
- Trewin, B.J., Darbro, J.M., Jansen, C.C., Schellhorn, N.A., Zalucki, M.P., Hurst, T.P., and Devine, G.J. (2017). The elimination of the dengue vector, *Aedes aegypti*, from Brisbane, Australia: The role of surveillance, larval habitat removal and policy. *PLoS Negl Trop Dis* 11, e0005848.
- Trewin, B.J., Pagendam, D.E., Johnson, B.J., Paton, C., Snoad, N., Ritchie, S.A., Staunton, K.M., *et al.* (2021). Mark-release-recapture of male *Aedes aegypti* (Diptera: Culicidae): Use of rhodamine B to estimate movement, mating and population parameters in preparation for an incompatible male program. *PLoS Negl Trop Dis* 15, e0009357.
- Tripet, F., Lounibos, L.P., Robbins, D., Moran, J., Nishimura, N., and Blosser, E.M. (2011). Competitive reduction by satyrization? Evidence for interspecific mating in nature and asymmetric reproductive competition between invasive mosquito vectors. *Am J Trop Med Hyg* 85, 265-270.
- Tun-Lin, W.K., B.H.; Barnes, A. (1999). Interspecific Association Between *Aedes aegypti* and *Aedes notoscriptus* in Northern Queensland. *Dengue bulletin* 23.
- Uemura, N., Itokawa, K., Komagata, O., and Kasai, S. (2024). Recent advances in the study of knockdown resistance mutations in *Aedes* mosquitoes with a focus on several remarkable mutations. *Curr Opin Insect Sci* 63, 101178.

Uetz, P., Melnik, S., Grünwald-Gruber, C., Strasser, R., and Stoger, E. (2022). CRISPR/Cas9-mediated knockout of a prolyl-4-hydroxylase subfamily in *Nicotiana benthamiana* using DsRed2 for plant selection. *Biotechnology Journal* 17, 2100698.

US Centers for Disease Control and Prevention (2022). Life stages of *Ae. aegypti* and *Ae. albopictus* mosquitoes (USA).

US Environmental Protection Agency (2020). Human health and environmental risk assessment for the new product OX5034 containing the tetracycline-repressible transactivator protein variant (tTAV-OX5034; new active ingredient) protein, a DsRed2 protein variant (DsRed2-OX5034; new inert ingredient), and the genetic material (Vector pOX5034).

van den Hurk, A.F., Nicholson, J., Beebe, N.W., Davis, J., Muzari, O.M., Russell, R.C., Devine, G.J., *et al.* (2016). Ten years of the Tiger: *Aedes albopictus* presence in Australia since its discovery in the Torres Strait in 2005. *One Health* 2, 19-24.

Viennet, E., Frentiu, F.D., McKenna, E., Torres Vasconcelos, F., Flower, R.L.P., and Faddy, H.M. (2024). Arbovirus Transmission in Australia from 2002 to 2017. *Biology* 13, 524.

Watkinson, A.J., Murby, E.J., Kolpin, D.W., and Costanzo, S.D. (2009). The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Sci Total Environ* 407, 2711-2723.

Webb, C.D., S.; Russell, R. (2016). A guide to mosquitoes of Australia (Australia: CSIRO publishing).

Whittle, L., Chapman, R., and Williamson, A.L. (2023). Lumpy Skin Disease-An Emerging Cattle Disease in Europe and Asia. *Vaccines (Basel)* 11.

WHO (2016a). Monitoring and managing insecticide resistance in *Aedes* mosquito populations - Interim guidance for entomologist.

WHO (2016b). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (Second edition).

WHO (2022a). Determining discriminating concentrations of insecticides for monitoring resistance in mosquitoes: report of a multi-centre laboratory study and WHO expert consultations.

WHO (2022b). Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO tube tests.

Wong, W.J., Tan, C.H., Verkaik, M.G., Ng, L.C., Hoffmann, A.A., and Chong, C.S. (2025). Suppression of *Aedes aegypti* may not affect sympatric *Aedes albopictus* populations: findings from two years of entomological surveillance in Singapore. *Sci Rep* 15, 2253.

Yen, P.S., and Failloux, A.B. (2020). A Review: Wolbachia-Based Population Replacement for Mosquito Control Shares Common Points with Genetically Modified Control Approaches. *Pathogens* 9.

Zhou, J., Liu, S., Liu, H., Xie, Z., Liu, L., Lin, L., Jiang, J., *et al.* (2022). Interspecific mating bias may drive *Aedes albopictus* displacement of *Aedes aegypti* during its range expansion. *PNAS Nexus* 1, pgac041.

Zhu, Z., Zheng, T., Lee, C.G., Homer, R.J., and Elias, J.A. (2002). Tetracycline-controlled transcriptional regulation systems: advances and application in transgenic animal modeling. *Semin Cell Dev Biol* 13, 121-128.

## Appendix A: Summary of submissions

The Regulator received several submissions from prescribed experts, agencies and authorities<sup>3</sup> on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	No comments but interested in receiving updates and reports on the progress and outcomes.	Noted.  The RARMP will be sent out for consultation to the public, prescribed agencies, state and territory contacts and local government areas.
2	<p>Noted that:</p> <ul style="list-style-type: none"> <li>At first, thought it was a good proposal but would be more effective if the modification affected both male and female populations.</li> <li>Continued release is needed creating a demand and commercial market for the GM mosquitoes.</li> <li>The proposal may not be effective in the permanent reduction of numbers of <i>Ae. aegypti</i> mosquitoes in the wild.</li> </ul> <p>Several questions were raised as listed below:</p> <ul style="list-style-type: none"> <li>Will male mosquitoes interbreed with other species due to the reduction in females of the same species to mate with, which could lead to hybrids that can potentially carry dengue fever?</li> </ul>	<p>Noted.</p> <p>The OGTR is only able to assess the application as presented and cannot compare with other techniques or consider the effectiveness of the GMO as discussed in Chapter 1, Section 1.1 of the RARMP.</p> <p>As addressed in Risk scenario 6, it is highly unlikely that the GM <i>Ae. aegypti</i> could interbreed with other species of mosquitoes to produce viable offspring. Therefore, it is unlikely that it could lead to hybrids that can carry dengue virus.</p>

<sup>3</sup> Prescribed experts, agencies and authorities include GTTAC, State and Territory Governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	<ul style="list-style-type: none"> <li>Could this then lead to dengue fever seen in other genus of mosquitoes?</li> </ul>	As mentioned above, it is unlikely that interbreeding would produce viable offspring. Hence, it is unlikely that other genus of mosquitos will be able to carry dengue.
3	<p>No official policy on genetically modified products or trials.</p> <p>Noted that:</p> <ul style="list-style-type: none"> <li>the release is in Queensland and not in their local government area.</li> <li>if the GM mosquito strain is proven to be safe and poses no threat to the greater community, the council would have no objections to its trial use, especially if it is proven to reduce the population of mosquitoes responsible for the transmission of dengue.</li> </ul>	<p>Noted.</p> <p>The Regulator is consulting all LGAs as it is a commercial application.</p> <p>The Regulator is preparing a RARMP that takes into account all scientific evidence to ensure the safety of people and the environment.</p>
4	Referred matter to the Environmental Health Team for review and follow up and a full response will be sent in due course	Noted.
5	<p>Noted the following:</p> <ul style="list-style-type: none"> <li>Male mosquitoes do not bite, and female mosquitoes are proposed to not survive.</li> <li>That the GM mosquito will be available to the general public and GM mosquitoes may not be confined to the release sites.</li> <li>Mosquitoes (in all life stages) can be food for other organisms.</li> <li>The applicant must apply to include <i>Ae. aegypti</i> in the Live Import List before the species is imported for commercial release. This includes a rigorous assessment of the impact on the Australian environment and native species, takes 18-24 months, and conditions may apply to the specimens.</li> <li>If there are valid concerns regarding possible impacts to food chain may need</li> </ul>	Noted.

Submission	Summary of issues raised	Comment
	to refer the proposal under the <i>Environment Protection and Biodiversity Conservation Act 1999</i> (EPBC Act).	
	Advised to consider the following in preparation of the RARMP:	
	<ul style="list-style-type: none"> <li>Survival rate of females carrying the modified genes and implications if they do survive, including risk associated with surviving females biting humans or other animals and appropriate risk management measures;</li> </ul>	The survival rate of wild type and GM mosquitoes are discussed in Section 4.2.1 of the RARMP.
	<ul style="list-style-type: none"> <li>Potential impacts on other organisms if GM mosquitoes (in any stage of life) are consumed;</li> </ul>	The potential impact on other organisms in the food chain are discussed in risk scenarios 4 and 6.
	<ul style="list-style-type: none"> <li>Impacts on the food chain compared to broadcast pesticide use;</li> </ul>	The impacts on the food chain and reference to pesticide use are discussed in risk scenario 6.
	<ul style="list-style-type: none"> <li>Risk to other mosquito species from potential interbreeding;</li> </ul>	The risk of interbreeding with other species of GM mosquitoes are discussed in risk scenario 6.
	<ul style="list-style-type: none"> <li>Cumulative impacts, potential risks (if any) of overstocking and optimum stocking density and assumption that insects can move around the landscape with water and optimum frequency of release;</li> </ul>	The commercial release of the GM mosquitoes in Brazil and field trials in Brazil and USA, which would have covered a large area has not demonstrated any negative ecological impacts and are discussed in risk scenario 6.
	<ul style="list-style-type: none"> <li>If there is a trial period rather than a general release, could Wetlands of International Importance (Ramsar sites) be excluded from release areas.</li> </ul>	This is an application for commercial release of the GM mosquitoes in Queensland. The intention is to release the GM mosquitoes in areas that <i>Ae. aegypti</i> is present. As mentioned in Chapter 1, Section 3.1,



Submission	Summary of issues raised	Comment
		<i>Ae. aegypti</i> typically breed indoors or in artificial containers outside houses and are typically found in association with humans. They are not known to breed in natural wetlands such as the 5 Wetlands of International Importance (Ramsar sites) in mainland Queensland.
	<ul style="list-style-type: none"> <li>Questions the benefits of the technology given the success of previous trials and wants the applicant to demonstrate that the benefits outweigh the risks;</li> </ul>	The OGTR does not carry out a risk/benefit analysis of the proposed dealings as discussed in Chapter 1, Section 1.1 of the RARMP.
	<ul style="list-style-type: none"> <li>The need to appropriately inform and consult First Nations communities;</li> </ul>	The RARMP will be out for public consultation as part of the <i>Gene Technology Act 2000</i> .
	<ul style="list-style-type: none"> <li>Risk associated with genetic modification;</li> </ul>	The RARMP has assessed the risks to people and the environment associated with the GMO and determined that these are negligible.
	<ul style="list-style-type: none"> <li>Health monitoring to be available for those exposed;</li> </ul>	Therefore, no conditions are imposed regarding the health monitoring of people.
	<ul style="list-style-type: none"> <li>GM mosquitoes may carry and develop unknown pathogens;</li> </ul>	As mentioned in Chapter 1, Section 2.1 the GM mosquitoes have been tested to ensure the absence of any arboviruses prior to import into Australia.
	<ul style="list-style-type: none"> <li>Impact on native species reliant on mosquitoes as a food source;</li> </ul>	The impact on native species reliant on mosquitoes and the survival and persistence of GM mosquitoes have
	<ul style="list-style-type: none"> <li>Survival and persistence of GM mosquitoes.</li> </ul>	

Submission	Summary of issues raised	Comment
	<p>Additional comments:</p> <ul style="list-style-type: none"> <li>Amendment to corresponding legislation for accuracy.</li> <li>To discuss impacts on the food chain, noting the vast improvements compared to pesticide use.</li> </ul>	<p>been addressed in risk scenarios 4 to 6.</p> <p>Amended.</p> <p>The potential impact on other organisms in the food chain are discussed in risk scenarios 4.</p> <p>The impacts on the food chain in reference to pesticide use are discussed in risk scenario 6.</p> <p>The GM mosquitoes are also not toxic as discussed in risk scenarios 4 and 5.</p>
6	<p>Noted that:</p> <ul style="list-style-type: none"> <li>Only males are released, and males do not bite and there is no identifiable direct human risk.</li> <li>Modification prevents survival of females and there will be no uncontrolled increase in the environmental population of <i>Ae. aegypti</i>.</li> <li>Theoretically possible that female GM larvae could survive if they develop in water with sufficient amount of tetracycline (e.g. waste water treatment plants or rural effluents where tetracycline is administered to agricultural livestock).</li> </ul> <p>The RARMP should consider the following:</p> <ul style="list-style-type: none"> <li>Restricting the release of the GM <i>Ae. aegypti</i> to areas away from tetracycline contamination sites.</li> <li>GM mosquitoes should not be released where <i>Ae. aegypti</i> is not already present whether or not dengue outbreaks occur.</li> </ul>	<p>Noted.</p> <p>The RARMP considered the risk of persistence of GM <i>Ae. aegypti</i> in the presence of tetracycline in the environment in Risk scenarios 1 and 2.</p> <p>The OGTR does not carry out a risk/benefit analysis of the proposed dealings as discussed in Chapter 1,</p>

Submission	Summary of issues raised	Comment
	<ul style="list-style-type: none"> <li>Address and manage any risk of insecticide resistance that may develop in wildtype <i>Ae. aegypti</i>.</li> <li>Contingencies to limit and mitigate the spread of the GM <i>Ae. aegypti</i> post-release should unintended adverse environmental consequences be identified.</li> <li>Considerations on undertaking ecological modelling to explore the consequences to other native fauna that feed on mosquitoes and the broader risk to species biodiversity, of significant fluctuations in <i>Ae. aegypti</i> population from the repeated releases of GM <i>Ae. aegypti</i>.</li> <li>The impact of the release of the GM <i>Ae. aegypti</i> on the other control strategies currently used (e.g. <i>Wolbachia</i>).</li> <li>Unintended increase in population of other mosquitoes (e.g. <i>Culex spp</i>) due to reduced interspecific competition and how it will be managed.</li> <li>Introgression of non-transgene genetic material into the genetic background of the WT population and whether any long-term genetic monitoring to screen for introgression.</li> </ul>	<p>Section 1.1 of the RARMP.</p> <p>The GM mosquitoes have been characterised to not contain common insecticide resistant genes (Chapter 1, Section 4.2.9).</p> <p>Current surveillance and mosquito control measures are in place (e.g. insecticides) and can be used to should any adverse environmental consequences be identified.</p> <p>Predators are not solely reliant on <i>Ae. aegypti</i> as a food source and mosquitoes are not known as an essential pollinator. <i>Ae. aegypti</i> is also only one of many species of mosquitoes in Australia.</p> <p>The OGTR does not carry out a risk/benefit analysis of the proposed dealings as discussed in Chapter 1, Section 1.1 of the RARMP.</p> <p>The presence of other <i>Aedes</i> mosquitoes and other species of mosquitoes in Australia has been discussed in Chapter 1, sections 5.3 and 5.4 respectively.</p> <p>The risk of the potential changes in the dynamics of mosquito populations have been addressed in risk scenario 6.</p> <p>The OGTR considers risks posed by the genetic modification. Risks from introgression of the background of the GM mosquito are outside the</p>

Submission	Summary of issues raised	Comment
	<ul style="list-style-type: none"> <li>Likelihood of horizontal gene transfer to <i>Ae. aegypti</i> associated microbes and/or other insect species.</li> </ul>	<p>scope of the OGTR assessment.</p> <p>The pathway for HGT gene transfer to occur is highly unlikely and explained in Chapter 2, section 2.2.</p>
7	<p>Have consulted experts within the State Government and do not have specific advice on the development of the RARMP at this stage.</p> <p>Will comment on the RARMP when it is released for comments.</p> <p>The agency has sought clarification on the following:</p> <ul style="list-style-type: none"> <li>The remit and responsibilities of other Regulatory agencies that are regulating the application.</li> <li>APVMA registration process and conditions.</li> <li>The potential for mosquito boxes to become breeding ground for other mosquitoes if they are not recovered.</li> <li>Tracking of boxes.</li> <li>Risk of GM mosquitoes to move into areas they have not been designated or assessed for (e.g. Torres Straits islands and Papua New Guinea)</li> <li>Restriction of authorisations for supply and deployment to suitably qualified</li> </ul>	<p>Noted.</p> <p>The remit and responsibilities of other Regulatory agencies is described in Chapter 1 Section 1.1 and throughout the RARMP.</p> <p>The OGTR cannot comment on the processes of the APVMA. Please contact the APVMA for further information.</p> <p>Labelling instruction would be handled by the APVMA if the product is registered.</p> <p>The OGTR only considers risk resulting from the GM mosquitoes. Other Agencies may consider that aspect and add disposal instruction to those rearing boxes.</p> <p>The OGTR may include licence conditions regarding the location where the product can be available for sale.</p> <p>The OGTR can only regulate dealings conducted within Australia.</p> <p>The OGTR will be drafting licence conditions and</p>

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	state or local government employees (e.g. those currently undertaking mosquito control activities).	consider where the product should be available for sale if a risk has been identified.
	Agency has notified the legislative framework to manage potential authorisation by Queensland.	Noted.
	The agency:	
	<ul style="list-style-type: none"> <li>Sought clarification on the roles and responsibilities in relation to the benefits of the technology.</li> </ul>	<p>The remit and responsibilities of other Regulatory agencies is described in Chapter 1 Section 1.1 and throughout the RARMP.</p> <p>The OGTR does not carry out a risk/benefit analysis of the proposed dealings.</p>
	Suggested that the RARMP considers:	
	<ul style="list-style-type: none"> <li>The impact on Wolbachia mosquitoes.</li> </ul>	The impact on Wolbachia mosquitoes have been discussed in risk scenario 3 of the RARMP.
	<ul style="list-style-type: none"> <li>The impact on existing public health measures.</li> </ul>	The OGTR does not carry out a risk/benefit analysis of the proposed dealings as discussed in Chapter 1, Section 1.1 of the RARMP.
	<ul style="list-style-type: none"> <li>The changes in public attitudes and behaviour in controlling mosquitoes.</li> </ul>	This is outside of the OGTR's remit. The OGTR only carries out a scientific evaluation of the risks to human health and the environment based on the genetic modification.
	<ul style="list-style-type: none"> <li>The genetic background of the parent organism used to generate the GM mosquito.</li> </ul>	This is outside of the OGTR's remit and would be considered by the APVMA (quality), DAFF and DCCEEW (import). However, as described in Section 2.1, the mosquitoes are tested to ensure the absence of arboviruses.

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	<ul style="list-style-type: none"> <li>Rationale of making the technology only available in Queensland.</li> </ul>	<p>The aim of the technology is to reduce the population of <i>Ae. aegypti</i> by interbreeding of the GM mosquitoes with wild type mosquitoes.</p> <p><i>Ae. aegypti</i> is currently only found in the state of Queensland.</p>
	<ul style="list-style-type: none"> <li>How the GM mosquitoes would be limited to Queensland.</li> </ul>	<p>The GM mosquitoes are intended to be released where <i>Ae. aegypti</i> is present. Only male mosquitoes would be released and if there are not wild type mosquitoes present in the release area, the GM male mosquitoes will quickly die out and will not be able to persist.</p>
	The agency:	
	<ul style="list-style-type: none"> <li>Clarified their responsibilities in the authorisation of the release of the GMO in Australia.</li> </ul>	Noted.
	<ul style="list-style-type: none"> <li>Notified of the change in the Department's name.</li> </ul>	Noted.
8	<p>No concerns on the risks associated with the application.</p> <ul style="list-style-type: none"> <li>The GM mosquitoes are self-limiting;</li> <li>The transgenes have been widely used;</li> <li>Similar idea to sterile fruit fly and sheep strike fly trial 30 years ago;</li> </ul> <p>Stated that the effectiveness of reducing dengue has not been demonstrated and that the release of Wolbachia in Northern Queensland has helped to eradicate local transmission of dengue.</p>	<p>Noted.</p> <p>The quality and efficacy of a pest control agent is outside of the scope of the Regulator's assessment and will be considered by the APVMA.</p>
9	Suggested changes to align with the terminology used in their current legislation.	Amended.
10	Recommendations	

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	<p>The committee agrees that the following should be included in the RARMP:</p> <ul style="list-style-type: none"> <li>• Potential for harm due to accidental exposure of humans and animals to the GMO including the impact of the expression of foreign protein on animals feeding on mosquitoes;</li> <li>• Potential for persistence of the GMO in the environment; and</li> <li>• Potential for the GMO to be harmful to the environment, including the potential harm to other mosquito species in the environment and the impact on animals or plants relying on these mosquitoes for their survival.</li> </ul> <p>The committee recommends that the Regulator should consider potential for crossbreeding with other mosquitoes.</p> <p>The committee advises to consider the following:</p> <ul style="list-style-type: none"> <li>• The impact of the GM mosquito release on the population of mosquitoes carrying <i>Wolbachia</i> and any subsequent health risks to people from dengue.</li> <li>• Consider the incidence of arbovirus in the environment.</li> <li>• Consistency of wording around how long the GM mosquitoes will persist in the environment.</li> <li>• Resolve the number of dengue cases in the different jurisdictions.</li> </ul>	<p>The potential for harm due to accidental exposure of humans and animals to the expression of foreign proteins have been considered in risk scenarios 1, 4 and 5.</p> <p>The potential for persistence of the GMO in the environment has been considered in risk scenarios 1, 2 and 5.</p> <p>The potential harm to the dynamics of the ecosystem has been considered in Risk Scenario 6.</p> <p>The potential for crossbreeding has been considered in risk scenario 6.</p> <p>The impact of the GM mosquito release on the population of mosquitoes carrying <i>Wolbachia</i> has been considered in risk scenario 3.</p> <p>Additional impact of the release has been added to risk scenario 3.</p> <p>Clarified that the low numbers of arboviruses relate to arboviruses that are carried by <i>Ae. aegypti</i> and not arboviruses in general.</p> <p>Text has been amended for consistency.</p> <p>Numbers and text have been corrected</p>

