Australian Government
Department of Health
Office of the Gene Technology Regulator

5 March 2018

Risk Assessment and Risk Management Plan

(consultation version)

for

DIR 159 – Limited and controlled release of genetically modified insect-specific viruses as vaccines against *Kunjin virus* infection in farmed crocodiles

Applicant – The University of Queensland

This RARMP is open for consultation until 10 April 2018.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

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

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

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

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Summary of the Risk Assessment and Risk Management Plan (Consultation Version)

for

Licence Application No. DIR 159

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. It qualifies as a limited and controlled release application under the *Gene Technology Act 2000* (the Act). The University of Queensland (UQ) proposes to conduct field trials to assess the efficacy and safety of two GMO vaccines for protection of farmed crocodiles from *Kunjin virus* infection.

Veterinary medicines must be approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA), which provides a national registration scheme for agricultural and veterinary chemical products under the *Agricultural and Veterinary Chemicals Code Act 1994* (AgVet Code), including vaccines. Therefore, in addition to approval by the Regulator, UQ would require a permit from APVMA to supply and use the GM vaccine for the purpose of animal research.

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

The application

Application number	DIR 159
Applicant:	The University of Queensland
Project Title:	Limited and controlled release of genetically modified insect-specific viruses as vaccines against *Kunjin virus* infection in farmed crocodiles [1]
Parent organism:	Two insect-specific flaviviruses, ISFa and ISFb[2]
Modified genes:	Insertion of two genes from a naturally attenuated strain of *Kunjin virus*[2]
Proposed release date:	Once all the required approvals have been granted
Proposed duration:	5 years

[1] The title of the project as submitted by the applicant is "Recombinant insect-specific viruses as non-infectious vaccines against *Kunjin virus* infection in farmed crocodiles".

[2] The specific details relating to the identity of the parent organisms, the design, construction and genetic modifications of the GMO, including the *Kunjin virus* genes, corresponding proteins and their function, are under consideration as Confidential Commercial Information (CCI) under section 185 of the Act. Any confidential information will be made available to the prescribed experts and agencies.

Proposed locations:	Two crocodile farms in Litchfield Council in the Northern Territory
Primary purpose:	To study the safety and efficacy of two genetically modified insect-specific viruses as vaccines against *Kunjin virus* infection in farmed crocodiles

Kunjin virus is a mosquito-borne virus endemic in the Northern Territory. Its primary host is birds but it also infects and causes disease in other animals (in particular, horses) and people. In crocodiles*, Kunjin virus* infection is largely non-symptomatic but may result in the development of skin lesions, which interfere with subsequent processing for leather and leather goods manufacturing. The proposed field trials would take place at the Darwin Crocodile Farm, Bees Creek, Northern Territory; and Janamba Crocodile Farm, Middle Point, Northern Territory and involve inoculation of up to 2,800 juvenile crocodiles with the GMO vaccines. Crocodiles would be harvested approximately 18-30 months after inoculation, and processed for crocodile products on site at the crocodile farms. As is common in veterinary vaccine trials, the products of vaccinated crocodiles could enter general commerce, including use in human food or animal feed.

Risk assessment

The risk assessment concludes that risks to the health and safety of people and the environment from the proposed release are negligible. No specific risk treatment measures are required to manage these negligible risks.

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

Credible pathways to potential harm that were considered included exposure of people or insects to the GMO and the potential for recombination with other viruses. Potential harms that were considered in relation to these pathways included adverse immune response, increased disease in people or animals, and impacts on insect biodiversity.

The principal reasons for the conclusion of negligible risks are the phenotype of the GMOs, in particular their limited host range and lack of ability to replicate in vertebrates, and suitability of the controls proposed by the applicant.

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the draft licence includes limits on the size, location and duration of the release, as well as a range of controls to minimise the potential for the GMO to spread in the environment. In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

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Abbreviations

AgVet Code	*Agricultural and Veterinary Chemicals Code Act 1994*
APVMA	Australian Pesticides and Veterinary Medicines Authority
C	*Flavivirus* capsid protein
°C	Degrees Celsius
CCI	Confidential commercial information
DAWR	Department of Agriculture and Water Resources
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
DENV	*dengue virus*
E	*Flavivirus* envelope protein E
ELISA	enzyme-linked immunosorbent assay
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GMO	genetically modified organism
ISF	insect-specific flavivirus
IBC	Institutional biosafety committee
KUNV	*Kunjin virus*
meter	m
microgram	µg
MODV	*Modoc virus*
NLRD	Notifiable Low Risk Dealings
NS	Non-structural
NKV	No Known Vector flaviviruses
OGTR	Office of the Gene Technology Regulator
ORF	Open reading frame
PCR	polymerase chain reaction
PPP	Primary Production and Processing
prM	*flavivirus* pre-membrane protein
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RT-PCR	Reverse transcription polymerase chain reaction
TGA	Therapeutic Goods Administration
the Act	*Gene Technology Act 2000*
WNV	*West Nile virus*
UQ	The University of Queensland
UTR	untranslated region

Chapter 1 **Risk assessment context**

Section 1 Background

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

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

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

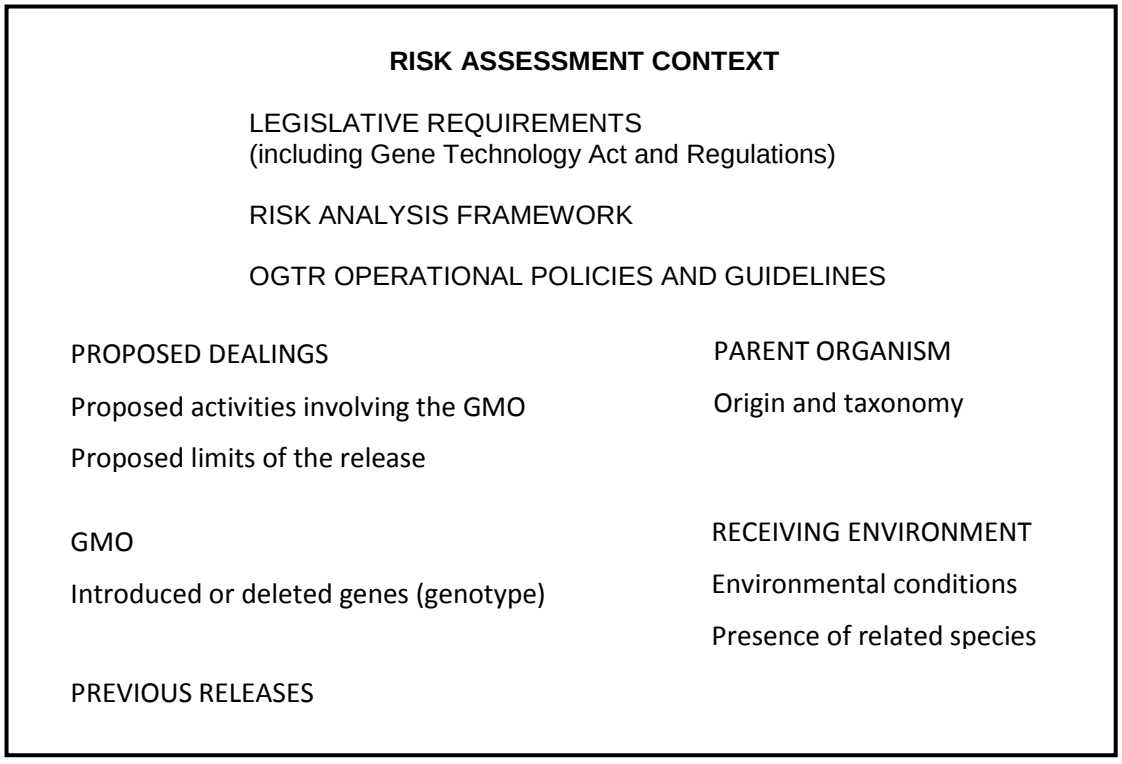

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, location and duration of the release, as

well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

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

7. The *Risk Analysis Framework* (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

2.1 Interface with other regulatory schemes

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

9. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies are generally not assessed by the Regulator.

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

11. As part of the permit process, the APVMA assesses the quality, safety and efficacy of the vaccine. Quality aspects could include batch-to-batch consistency in vaccine composition, purity and potency. The APVMA audits the Good Manufacturing Practice record of the applicant. Safety aspects include the toxicological profile of the vaccine and its residues, including metabolites and degradation products. Associated food safety risks and consumer dietary exposure is also considered by the APVMA. The APVMA approves the label, handling and directions for use of veterinary vaccines to ensure safe use. The APVMA may also impose conditions on a permit for the use of veterinary vaccines for research purposes.

12. FSANZ develops the food standards in the Food Standards Code with advice from other government agencies and input from stakeholders. The Standards in the Food Standards Code are legislative instruments and the Food Standards cover the composition of some foods, such as dairy, meat and beverages. FSANZ is also responsible for labelling of packaged and unpackaged food, including specific mandatory warnings or advisory labels.

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

14. FSANZ has developed the Primary Production and Processing (PPP) Standard for Meat and Meat Products (FSANZ, 2010), which includes meat from farmed crocodiles. PPP Standards (which only apply in Australia) aim to strengthen food safety and traceability throughout the food supply chain

from paddock to plate. The standard introduces legal safeguards if there is a food-related adverse incident, allowing regulators to investigate food safety matters through the entire meat supply chain.

15. The processing of crocodile meat is covered by the Australian Standard: AS 4467-1998 *Hygienic Production of Crocodile Meat for Human Consumption*. This standard applies to the construction of all premises where crocodiles are slaughtered and processed, and equipment and procedures used in production of crocodile meat for human consumption.

Section 3 The proposed field trials

16. The University of Queensland (UQ) proposes to conduct field trials using live genetically modified (GM) insect-specific flaviviruses (ISFs) containing two genes from an attenuated *Kunjin virus* (KUNV). The aim of the trial is to assess the efficacy and safety of the GM ISFa-KUN and ISVb-KUN as GMO vaccines for protection of crocodiles from KUNV infection.

17. The parent organisms for the GMOs are two distinct insect-specific flaviviruses (ISFs – ISFa and ISFb) normally associated with mosquitoes in northern Australia. They are not known to cause disease in infected mosquitoes and are not able to replicate or cause disease in vertebrate animals.

18. In the GMOs, two genes that encode virion proteins of ISFa and ISFb have been replaced by the corresponding genes of KUNV. The GMOs are intended to stimulate an immune response against KUNV in inoculated crocodiles. Like the parent viruses, the GMOs are incapable of reproduction invertebrates.

19. The dealings assessed by the Regulator are:

- conduct of experiments with the GMOs;
- transporting the GMOs;
- disposing of the GMOs; and

the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

3.1 The proposed limits of the field trials (duration, scale, location and people)

20. The field trials are proposed to take place over a 5 year period from the date of issue of the licence. Up to 2800 crocodiles are expected to be inoculated with either of the two GMOs.

21. The crocodile farms proposed to participate in the field trials are the Darwin Crocodile Farm, Bees Creek, Northern Territory (NT); and Janamba Crocodile Farm, Middle Point, Northern Territory.

3.2 The proposed controls to restrict the spread and persistence of the GMO in the environment

22. The applicant has proposed a number of controls to restrict the spread and persistence of the GMO in the environment. These include:

- administration of the GMO by appropriately trained farm personnel or qualified veterinarian in accordance with trial protocols
- following biosecurity measures for crocodile farms in the Northern Territory, including:
  - keeping vaccinated crocodiles physically separated from non-vaccinated crocodiles for at least 4 weeks after the administration of the last dose of GMO
  - implementing crocodile pen entry and exit procedures, including wearing designated clothing and footwear, and the use of disinfectant footbaths
- restricting access to the vaccinated crocodiles to authorised persons only

- securing access to the vaccinated crocodiles using fences and locked gates with keypad access
- disposing deceased vaccinated crocodiles and waste following state and/or local council requirements
- decontaminating crocodile pens and equipment following state and/or local council requirements
- decontamination of GMO-contaminated materials using effective disinfectant
- transporting GMOs and tissue/blood samples taken from GMO-inoculated crocodiles for testing in accordance with the OGTR *Guidelines for the Transport, Storage and Disposal of GMOs*.

3.3 Details of the proposed activities

3.3.1 *Study design*

23. The GMOs will be administered to juvenile crocodiles (between 3 to 6 months old, weighing approximately 350 grams) by subcutaneous or intramuscular injection. Groups of 70 juvenile crocodiles will receive either a low dose (2 micrograms (µg), ~10^8 particles) or high dose (20 µg, ~10^9 particles) of one of ISFa-KUN or ISFb-KUN. Each crocodile will be inoculated 3 times at 4-week intervals. A maximum of 280 crocodiles will be inoculated with each GMO per year.

24. Approximately 30% (20 per group of 70 animals) of the vaccinated crocodiles will be tested for an antibody response to the GMOs and confirmation that the GMO has been eliminated from the body at 4 weeks after each inoculation (including immediately prior to the second and third inoculations), and 3 months after final inoculation (5 months after first inoculation). All vaccinated animals will again be sampled for testing at time of harvest (12-30 months after inoculation). Testing for the GMOs is detailed in section 3.3.5.

25. Inoculated crocodiles will be physically isolated from other crocodiles for the duration of the GMO trial up to and including 4 weeks after the last inoculation. After this time, the inoculated crocodiles will be released into common farm pens to be grown and processed as usual.

26. Adjacent to each pen of GMO-inoculated crocodiles, unimmunised crocodiles of the same age will be kept in pens as negative controls to confirm that no spread of the GMOs on the farm has occurred. The unimmunised crocodiles in adjacent pens will also serve as sentinels for natural Kunjin virus infection and will be tested for antibodies specific for wildtype *Kunjin virus* or specific for the GMOs. These sentinel animals will be sampled and tested for the presence of the GMOs or wildtype Kunjin virus as detailed in section 3.3.5.

27. Mosquitoes on the crocodile farms will be sampled and monitored for the presence of GMOs on a weekly basis over the period of the trial, as is detailed in section 3.3.5.

28. In addition, water from the vaccinated and control crocodile pens will be sampled daily over the period of the trial, as is detailed in section 3.3.5.

29. Vaccinated and sentinel crocodiles will have two metal engraved small animal tags for identification.

3.3.2 *Animal containment and housing*

30. The hatchling facilities/areas are separated from other areas of the crocodile farms by a boundary fence (1.8 m buried mesh fence). In addition, the crocodile farms have an outer boundary fence (1.8 m buried chain-mesh fence).

31. The buildings holding the hatchling/juvenile crocodiles at the crocodile farms are completely enclosed with concrete floors, brick walls and covered with fully enclosed roof to deter predatory birds.

32. The hatchling facilities are comprised of pens internally divided by walls that are sufficient to physically separate and contain the crocodiles, with grated drains to prevent crocodiles escaping.

33. The trial will occur on two farms with slightly different crocodile housing:

- Darwin Crocodile Farm – Crocodiles are stocked at 35 crocodiles/pen. Each pen is 116.5 cm wide and 209.5 cm long within a larger shed. The rear of each pen is covered to provide crocodiles a hiding area and the front of the pen has an open air feeding platform. Pen water is maintained at 32°C by an automated system that injects heated water into each pen. Excess water drains into a collection pond. This is estimated to result in a water change 3 times per day.
- Janamba Crocodile Farm – Crocodiles are stocked at 70 crocodiles/pen. Each pen is 156 cm wide x 301 cm long and completely enclosed. Pen water is heated to 33°C by coiled pipes within the water body of the pen.

34. At both farms, the crocodile pens contain approximately 100 litres (L) of water on average, and pens are completely drained into collection ponds (~500,000 L) five days per week, and pens cleaned using a chlorine-based detergent/disinfectant.

35. Pens are checked at least twice daily; dead crocodiles and food waste are removed from the pens as soon as possible.

3.3.3 *Manufacture, supply and storage of the GMO*

36. The GMOs would be manufactured at the University of Queensland under a Notifiable Low Risk Dealing authorisation assessed by The University of Queensland institutional biosafety committee (IBC).

37. The quality and identity of each batch of GMOs will be checked by:

- sodium dodecyl sulfate polyacrylamide gel electrophoresis to confirm the size of the GMO particles
- viral RNA sequencing to confirm the identity and genetic stability of the virus
- inoculation onto vertebrate cell cultures (crocodile, monkey, human and mouse lines) to confirm the lack of virus replication in vertebrate cells
- enzyme-linked immunosorbent assay (ELISA) using a panel of monoclonal antibodies to the KUNV proteins expressed in the GMOs.

38. The GMOs would be supplied as a frozen liquid in a sealed plastic screwcap vials, each vial contain approximately 10^{10} infectious units of live GMO, representing 10-100 vaccine doses. These would be transported from The University of Queensland to the crocodile farms in accordance with the OGTR *Guidelines for the Transport, Storage and Disposal of GMOs* using couriers.

39. The GMOs will be stored at the release sites within a freezer (-80°C) in a restricted area. The receipt of GMOS at the farm trial sites will be logged and details of all GMO storage, use and disposal will be recorded.

3.3.4 *Preparation and administration of the GMO*

40. Reconstitution of the GMOs would take place in a room adjacent to the shed where the crocodile pens are located. Preparation and vaccination would be conducted by appropriately trained farm/research personnel or a registered veterinarian.

41. Prior to administration, the GMOs would be thawed and reconstituted with buffer containing adjuvant. All required syringes would be filled with one of the GMOs and recapped using a safe needle recapping device to avoid accidental needle stick injury.

42. At pen-side, each animal will be restrained by a trained crocodile farm handler wearing puncture- resistant gloves. The GMOs will then be administered by subcutaneous or intramuscular injection into the tail. GMO administration will be performed by personnel wearing gloves, eye protection (safety glasses) and a puncture-resistant glove on the hand not holding the syringe.

43. Empty syringes will be disposed directly into sharps containers without recapping to avoid needle stick injury. Used sharps containers will be sealed and disposed of by a waste contractor.

3.3.5 *Sample collection*

44. Blood samples will be collected from vaccinated and sentinel crocodiles for testing as described in paragraph 24. The animals will be caught by hand using best practice methods according to internal standard operating procedures (SOP) for the crocodile farm. Each animal will be restrained by a trained crocodile farm handler wearing puncture-resistant gloves. Blood collection will be carried out according to published protocols (Myburgh et al., 2014) using a 23 gauge needle and stored within serum tubes. Syringes used for blood collection will be disposed of as described in paragraph 43. Blood Samples would be tested for the GMO or wild type KUNV by ELISA.

45. Mosquitos collected near the pens of the vaccinated crocodiles will be tested for the presence of the GMOs by GMO-specific reverse transcription polymerase chain reaction (RT-PCR) on virus expectorated in their saliva during sugar feeding (Hall-Mendelin et al., 2010).

46. To assess shedding of the GMOs from vaccinated crocodiles, daily water samples will be taken from all pens containing the vaccinated crocodiles and sentinel crocodiles. The samples will be assessed for the presence of the GMO RNA genome by RT-PCR.

47. All samples collected from the farm would be transported by courier as biological specimens, and in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.* Samples would be transported to The University of Queensland for analysis.

48. Sample analyses will be undertaken at The University of Queensland and would be conducted under a Notifiable Low Risk Dealing (NLRD) authorised by the University of Queensland IBC.

3.3.6 *Personal protective clothing*

49. Personnel working in crocodile hatchery sections of the farms will wear hatchery-designated boots and clothing. Disposable overalls and booties will be worn during all crocodile vaccination procedures.

50. Farm/research personnel or veterinarian preparing and administering the GMOs would wear eye protection (safety glasses) and gloves, as detailed in paragraph 42.

3.3.7 *Decontamination and disposal of the GMO and general biosecurity measures*

51. Following administration of the GMOs, used vials and other waste generated during the vaccination procedure (i.e. gloves, syringes and alcohol swabs etc.), and any unused GMOs will be decontaminated using 10% bleach, 70% ethanol or discarded into appropriate biohazard containers. Biohazard waste will be disposed of following the sites' procedures in accordance with Northern Territory laws that apply to the disposal of biologically hazardous waste.

52. After handling the GMOs, any work surfaces used will be decontaminated with an appropriate chemical disinfectant, following standard farm procedures (e.g. a chlorine-based detergent followed by spraying with a sanitising solution (F10SC).

53. Contaminated clothing will be laundered following standard farm procedures (e.g. hot (71°C) water with detergent and hot air drying). Disposable overalls and booties used during vaccination procedures will be disposed as biologically hazardous waste, as detailed in paragraph 51.

54. All crocodile carcasses and food waste are collected into closed, vermin-proof waste bins and disposed by deep burial as detailed above.

55. Footbaths filled with fresh disinfectant effective against the GMO are available at the entrance to each hatchling area. When entering and exiting the hatchling area, boots would be disinfected against the GMO.

56. At both crocodile farms, water from the crocodile pens is drained into collection ponds on site and the pens are cleaned using a chorine-based detergent on the morning after every feeding. Hatchling crocodiles are fed five times per week in the afternoon.

57. Cleaning equipment is specific to each pen or shed and the removal or cross-use of cleaning equipment indifferent pens/sheds is not permitted. Equipment used in the hatchery areas is hatchery specific and not to be taken out of the hatchery or used in other areas of the farms.

58. Further detail on crocodile farm biosecurity practice is provided below (Section 3.4).

3.3.8 *Training of personnel*

59. All farm personnel responsible for handling the GMOs, inoculated crocodiles and contaminated equipment would be trained in handling the GMO, the decontamination and disposal of the GMO in accordance with the farm protocols, any licence conditions imposed by the Regulator and any permit conditions imposed by the APVMA.

60. All farm personnel responsible for handling the syringes for inoculating the crocodiles with the GMOs or sampling the vaccinated or sentinel crocodiles will be trained in the handling of sharps.

3.3.9 *Contingency measures*

61. If the GMOs are found to persist in the vaccinated crocodiles beyond the anticipated time frame (i.e. if they are detected in samples taken 4 weeks post-inoculation), the following measures will be taken:

- any further vaccination of the crocodiles will cease
- the vaccinated animal groups will continue to be isolated from other animals and monitored fortnightly until the GMO is shown to have been eliminated from all crocodiles
- if the GMO is still detected in the crocodiles after the 5-6 month testing period, they will be euthanased and their carcasses disposed of as biohazard waste by deep burial.

62. If the GMOs are found in the control/sentinel crocodiles, the following measures will be taken:

- no further vaccination with the GMO will take place
- all vaccinated animals and all control animals in pens where animals have tested positive for the GMO will be culled and carcasses tested for evidence of GMO replication
- all other pens on the farm will be monitored for evidence of the GMO in a representative group of animals (10% in each pen), with weekly assessment for one month until there is no evidence of further presence of the GMO in farm animals.

63. If the GMOs are found in the mosquitoes near the vaccinated crocodile pens, the following measures will be taken:

- no further vaccination with the GMO will take place
- a concentrated barrier mosquito spraying program with synthetic pyrethrins will take place on the farm to minimise mosquito numbers
- representative animals from both GMO-inoculated and control pens will also be immediately sampled for evidence of GMO transmission.

3.3.10 *Record keeping*

64. The applicant will ensure that procedures are in place to account for all GMO stocks transported to the crocodile farms in the Northern Territory under the licence. The GMOs will be accounted for from transport to destruction, and records will be made available to the Regulator on request. Records of training of farm personnel involved in the trial and of ongoing monitoring and auditing of trial sites will also be made available to the Regulator on request.

3.3.11 *Fate of crocodiles after field trials*

65. After inoculated crocodiles reach the appropriate age for harvesting (12-30 months after inoculation), they would be processed on-site in the same way as for other commercial crocodiles at the farm sites.

3.4 Biosecurity

66. To assist in the risk assessment of the proposed field trials (discussed in Chapter 2), this section describes the relevant biosecurity standards for crocodile farms, and the territory and local council requirements and legislation.

3.4.1 *Crocodile farm biosecurity*

67. As part of current arrangements between the Northern territory government and industry, crocodile farms are expected to implement on-farm biosecurity programs according to *Biosecurity of NT Crocodile Farms – Hygiene Procedures and Biosecurity Concerns* (Simlesa, 2010) and follow them on a daily basis to reduce the risk of transmission of disease onto and between crocodile farms.

68. The applicant has indicated the participating farms follow the *Biosecurity of NT Crocodile Farms – Hygiene Procedures and Biosecurity Concerns* (Simlesa, 2010) guidelines. The majority of biosecurity requirements have been detailed in Section 3.3 – Details of the proposed activities. Additional hygiene and biosecurity measures not previously mentioned in Section 3.3 are outlined below.

Farm personnel and visitors

69. Access to the farm by visitors, truck drivers, delivery personnel and employees from other crocodile farms is to be minimised, and visitor access recorded.

Crocodile pens and water

70. Pens should have access to sun and shade, a dry landing area, enough water to submerge and sufficient space to avoid overcrowding. Pens need to be protected from dust, direct winds, vermin and wildlife entry and monitored for bacterial counts of pen water on a regular basis.

71. Water needs to be clean and fresh from bore or town supply. It is not advisable to use surface water supplies unless chlorinated or UV treated. Water needs to be chlorinated at 2-3 parts per million and at a temperature of 32^{o}C.

Cleaning of crocodile pens

72. Crocodile pens are to be cleaned and left to dry before the introduction of new hatchlings into the farm system.

73. Pens need to be cleaned after every feed night by removing old food, rinsing, disinfecting, scrubbing, and rinsing prior to refilling with water. On non-feed nights, water should be reduced, with pens cleaned/scrubbed and water re-filled. Pens need clean fresh water on a daily basis.

74. Walls, hide boards and the floor need to be scrubbed with disinfectant at every clean. All cleaning equipment should be stored off the floor by either hanging, on shelves or stored in a receptacle of some sort. One set of cleaning equipment should be designated to each pen/shed.

Animals at the farms

75. Crocodile hatchlings should be introduced into clean pens with no animals from previous years.

76. Dead animals should be removed as soon as possible to prevent potential contamination.

77. All animals gained, sold, processed or deceased need to be recorded on the farm records.

78. Daily records of deaths and illness should be used to observe and react to unusual trends. Unknown illness or usual deaths should be submitted to the veterinary laboratories for analysis.

79. Domestic stock and pets should not access the farm site or enter into pens/sheds.

Equipment, infrastructure and consumables

80. All chemicals and drugs used should be registered for use on crocodile farms or in the process of being approved. Non-veterinarian treatment or treatment directed by a veterinarian but not applied by a veterinarian must be entered in the farm records and kept for two years. This includes product name, usage dates, dosage administered, withholding period and treated animals identified.

81. All equipment and infrastructure should be checked regularly. All chemicals should be used before the expiry date, with clear correct identification and labelling of cleaning chemicals and other solutions used on the farm is desirable to prevent accidental consumption by employees.

3.4.2 *High level biosecurity*

82. In the event of an outbreak of disease, the *Biosecurity of NT Crocodile Farms – Hygiene Procedures and Biosecurity Concerns* (Simlesa, 2010) recommends the following measures:

- the Northern Territory Government and the Northern Territory crocodile industry must be immediately notified;
- limiting personnel from entering the farm unless absolutely essential;
- visitors entering farm must have a head to toe shower before and after the visit;
- used clothing and personal protective equipment must remain on property;
- any vehicle entering the property must be washed and disinfected before and after going onto the property. Vehicles should be disinfected inside as well. Vehicles not entering the farm but parked outside should also be washed and disinfected before visiting another farm; and
- animals, waste or products must not be moved off the property until disease status is clarified.

83. If the cause of the death is an Emergency Animal Disease, then the relevant Australian Veterinary Emergency Plan (Ausvetplan) would be activated and the appropriate authorities would be notified. Disposal of carcasses, used litter and feed, and decontamination of equipment, would be under the direct control of the Territory's Chief Veterinary Officer.

84. The *Biosecurity Incident Management System* (Group, 2012) provides guidance for the management of biosecurity incident response in Australia and can be applied to all biosecurity sectors. Typically the states and territories have primary responsibility for preparing and responding to biosecurity incidents within their borders. The DAWR has a role in providing national leadership and coordination in preparing for and responding to biosecurity incidents.

Section 4 Parent organisms

85. Consideration of the characteristics of the unmodified organisms provides a baseline for comparing the potential harm from dealings with GMOs. The parent organisms are two distinct insect-specific flaviviruses, ISFa and ISFb, normally associated with mosquitoes in northern Australia[2]. As such, general information concerning biological properties of viruses within the *Flavivirus* genus and the relevant biological properties the ISF parent organisms will be discussed. The ISFa and ISFb are not known to cause disease in infected mosquitoes and are not able to replicate in vertebrate animals.

86. Further information on the parent organisms is presented in the CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

4.1 Background - *Flaviviruses*

87. The *Flavivirus* genus of the *Flaviviridae* family encompasses a diverse array of over 70 viruses. Most flaviviruses [also referred to as arboviruses (arthropod borne viruses)] are considered to be dual-host viruses and are transmitted horizontally between a vertebrate hosts and an arthropod host (mosquito or tick) through biting. These dual-host flaviviruses can be further divided into two distinct classes, mosquito/vertebrate and tick/vertebrate viruses. Examples of mosquito/vertebrate flaviviruses include *Dengue virus* (DENV), yellow fever *Japanese encephalitis virus* and *West Nile virus* (WNV), all of which are human pathogens of global concern (Kuno et al., 1998). *Kunjin virus* is a mosquito/vertebrate endemic to mainland Australia and Papua New Guinea. Tick/vertebrate flaviviruses associated with serious human disease include tick-borne encephalitis virus, Langat virus and Powassan virus (Blitvich and Firth, 2017).

88. Humans are typically dead-end hosts for arboviruses, as the virus cannot replicate to high enough titres to reinfect the arthropods needed to continue the virus life cycle. Exceptions to this are yellow fever and dengue viruses, which require mosquito vectors but are sufficiently well adapted to humans that they don't depend on animal hosts.

89. There are some flaviviruses which are maintained by vertebrates only, commonly known as No Known Vector (NKV) flaviviruses. NKV flaviviruses include those isolated exclusively from rodents (e.g. Modoc virus; MODV) and those isolated exclusively from bats (e.g. Rio Bravo virus) (Leyssen et al., 2002; Volkova et al., 2012).

90. Similarly, there are ISFs which replicate only in mosquitoes and form a specific subgroup within the Flavivirus genus. The ISFs are unable to replicate in vertebrate cells, and cannot be transmitted via classical horizontal transmission to vertebrate hosts (Blitvich and Firth, 2015).

91. ISFs can be further separated into two clades, Lineage I (classical) ISFs form a distinct clade within the flavivirus genus, while Lineage II (dual-host affiliated) display an insect-specific phenotype but cluster phylogenetically with the dual-host flaviviruses (Blitvich and Firth, 2015).

4.2 Basic Biology

92. Flaviviruses are lipid-enveloped viruses that contain a single-stranded, positive-sense RNA genome of approximately 10-11 kb, encoding a single open reading frame (ORF) that is flanked by 5' and 3' untranslated regions (UTRs). Viral proteins are made directly from the template strand or positive sense RNA which is present in the viral capsid. The ORF encodes a large polyprotein that is co-

[2] The identity of the parent organisms are under consideration as Confidential Commercial Information (CCI) under section 185 of the Act. Any confidential information will be made available to the prescribed experts and agencies.

and post-translationally cleaved to generate three structural proteins, designated the capsid (C), premembrane/membrane (prM/M) and envelope (E) proteins, and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Rice et al., 1985). The structural proteins are encoded at the 5' end of the genome and non-structural proteins at the 3' end (Figure 2).

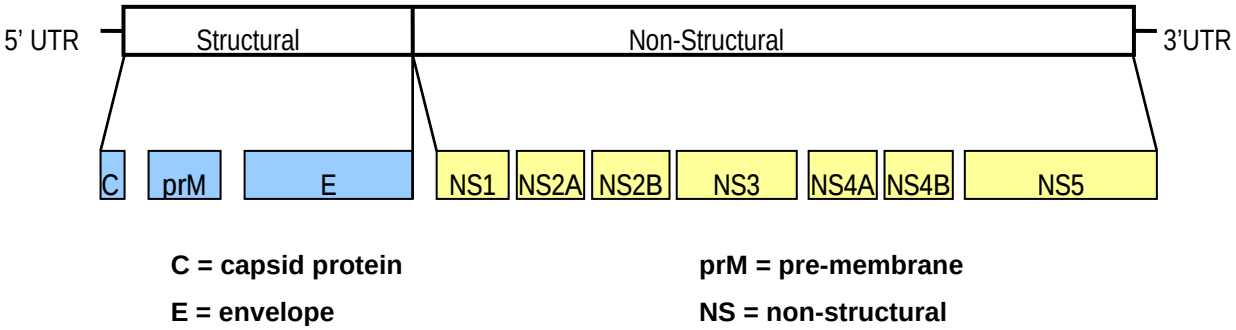

Figure 2 Genomic organisation of *Flavivirus* and encoded proteins

93. The proteins, as ordered from N-terminus of the polyprotein, are:

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

94. The seven non-structural proteins are primarily replicative proteins although some are also involved with confounding the host immune system (Munoz-Jordan et al., 2005). In addition to their primary role in viral replication, flavivirus NS2A, NS4A, NS4B and NS5 proteins are thought to inhibit interferon signalling and hence down-regulate the host antiviral immune response (Munoz-Jordan et al., 2005).

95. Flaviviruses have co-evolved with their host species and are generally host specific, and infect only certain tissue types within those organisms. Various studies have indicated the regions or proteins involved in host replication and/or specificity. These include the 5' untranslated region and amino acid substitutions in NS proteins 1 and 3 that were identified to be important for infectivity, transmissibility and replication of the Dengue-2 virus (DENV-2) vaccine candidate, primary dog kidney (PDK)-53 virus (Brault et al., 2011).

96. Studies with chimeric arboviruses and the NKV flavivirus MODV, found the inability of NKV viruses to infect and replicate in arthropod cells is not determined by the viral envelope proteins, but by a post-entry event (Charlier et al., 2010; Saiyasombat et al., 2014).

97. Similarly, a chimeric DENV genome carrying the envelope genes from Langat virus (LGTV), a tick-borne virus unable to infect mosquito cells, retained the ability to infect mosquito cells (Engel et al., 2011). These studies indicate that some molecular determinants of flavivirus tropism may be found outside of the prM-E structural region, such as in the capsid, NS2A, or NS4B proteins, regions that have previously been demonstrated to affect tick or mosquito tropism (McElroy et al., 2006; Schrauf et al., 2009).

4.2.1 *Flavivirus life cycle*

98. The life cycle of flaviviruses, like other viruses, involves the transmission of infective viral particles to a host organism; recognition, attachment and entry into the host cells; replication of viral nucleic acid and protein production; finally followed by assembly and release of infective viruses (see Figure 3).

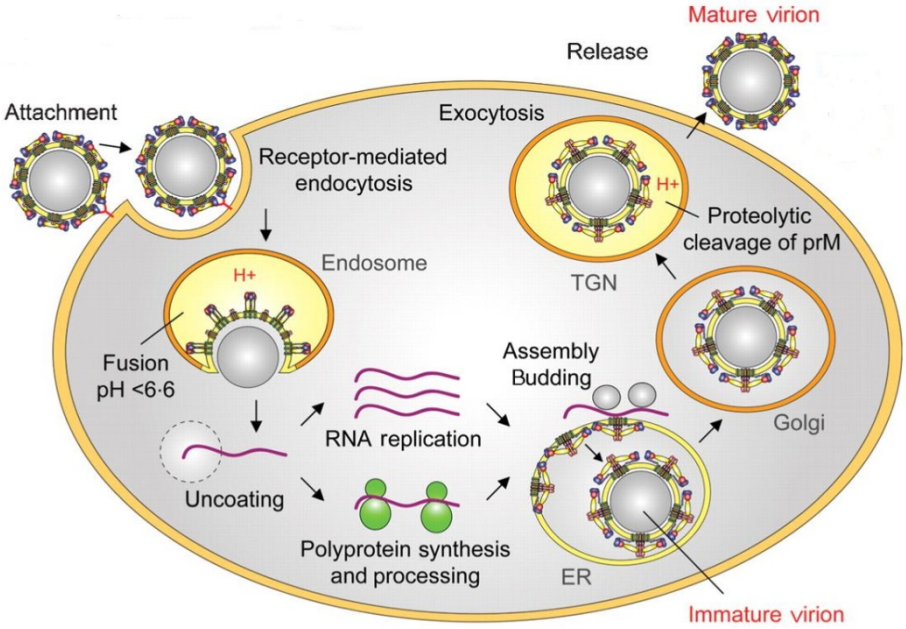

Figure 3 Steps in replication cycle of flaviviruses (Stiasny and Heinz, 2006)

99. Flaviviruses enter target cells by receptor-mediated endocytosis. The identity of the cellular receptors that mediate flavivirus entry and infection is, at present, poorly understood. A large number of molecules have been described as flavivirus candidate receptors in different cell types, however the identity of the cellular receptors that mediate flavivirus entry is an active area of investigation (Perera-Lecoin et al., 2013).

100. Once in the cell, flaviviruses are trafficked to early endosomes, where the acidic environment triggers major conformational changes in their envelope glycoprotein (E) protein that induce fusion of the viral and endosomal membranes, resulting in genome release (Modis et al., 2003; Bressanelli et al., 2004).

101. Replication of flaviviruses takes place in the cytoplasm. Flaviviruses cannot replicate in the nucleus because, like most other RNA viruses, it uses the host cell's RNA-dependant RNA polymerase to replicate. Genome replication in flaviviruses is carried out by a membrane-bound viral replication complex consisting of viral NS proteins, viral RNA and unidentified host proteins (Klema et al., 2015).

102. A full length copy of the complementary, minus strand genome is produced, and this complementary genome then serves as a template strand for further replication. After a ribosome binds to the genome, a single poly-protein is translated and then cleaved by a combination of viral and host proteases to release mature polypeptide products (Sun et al., 2017).

103. Assembly of new virions (see Figure 3, above) begins in the cytoplasm where viral RNA complexes with the C protein. This complex then associates with heterodimers of prM and E proteins in the ER membrane, and buds through the ER membrane into the ER lumen. The resulting immature, non-infectious virions have an ER-derived lipid bilayer carrying prM/E heterodimers. The immature virions are then transported to the trans-Golgi network where furin-mediated cleavage of prM to M generates mature infectious particles that are released by exocytosis (Stadler et al., 1997; Mackenzie and Westaway, 2001; Lorenz et al., 2003; Li et al., 2008; Yu et al., 2008).

4.3 Geographic Distribution and Natural Host range of ISFs

104. ISFs have a ubiquitous geographic distribution and have been isolated from mosquitoes in every continent with the exception of Antarctica. The mosquito host species specificity of ISFs appears to be virus specific (Colmant et al., 2017). Although ISFs have been isolated exclusively from mosquitoes, ISF-like sequences have been detected by molecular methods (e.g RT-PCR) in other dipterans (e.g. flies and midges) indicating that some ISFs may not have a mosquito-restricted host range [reviewed in (Blitvich and Firth, 2015)].

105. The ISFa and ISFb naturally infect mosquitoes that breed in fresh water habitats in Northern Australia. ISFa and ISFb were isolated from various regions of the Northern Territory, and are distributed in the Northern Territory and Kimberley region of Western Australia. ISFa and ISFb are known to be natural commensals of mosquitoes in Northern Australia and cannot replicate in vertebrates.

106. Further information on the geographic distribution of the parent organisms is presented in the CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

4.4 Transmission of ISFs

107. ISFs replicate exclusively in mosquitoes. They are thought to be transmitted vertically from an infected female to her progeny with no vertebrate intermediate (Lutomiah et al., 2007; Saiyasombat et al., 2011; Bolling et al., 2012).

108. Once infected with an ISF, a mosquito can transmit that virus for the rest of its life. The detection of ISFs in mosquitoes of all life stages, including adults of both sexes, indicates that vertical transmission is the primary mechanism by which these viruses persist in mosquitoes in nature [reviewed in (Blitvich and Firth, 2015)].

109. The study by Bolling *et al.* (Bolling et al., 2012) examined insect-specific flavivirus transmission dynamics in a naturally-infected mosquito colony, and found that vertical transmission was the primary method of virus transmission, with venereal (sexual) transmission potentially playing a minor role.

110. The ability of the ISFa parental virus to be transmitted horizontally to mosquitoes via a blood meal was examined. Mosquitoes fed 1000 infectious particles of ISFa in a blood meal did not become infected with the virus. Further information on these experiments is presented in the CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP. No specific information related to ISFb horizontal transmission was provided in the application.

111. ISFs have not been isolated from any vertebrates in nature, nor have they been found to replicate in any vertebrate cell line that has been tested (Blitvich and Firth, 2015). In spite of extensive knowledge of flavivirus replication, little is known about the viral proteins and the stages of the replication cycle involved in ISF host range restriction. Recent studies with the ISF *Niénokoué virus*, indicate that the ability of ISFs to infect vertebrates may be blocked at several stages of viral life cycle, including attachment/entry, RNA replication and at assembly/release (Junglen et al., 2017).

112. Analysis of the codon usage and dinucleotide bias in the genome of ISFs also reveals that they have genetically co-evolved with their insect hosts and are not optimised for replication or protein expression in vertebrate cells (Lobo et al., 2009).

113. To examine replication and transmissibility of the parental viruses in vertebrates, mice were inoculated with purified preparations of the parental viruses (1 mouse with ISFa and 1 mouse with ISFb). The mice were examined for an antibody response to a specific ISF protein that is indicative of a productive flavivirus infection and would be present in an infected host. There was no evidence of an antibody response to the specific ISF protein for the parental ISFs from the vaccinated mice. This experiment indicated that the parental ISFs are unable to infect mice (vertebrate host) resulting in a productive flavivirus infection.

114. Further information on transmission of the parent organisms is presented in the CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

4.5 Environmental stability and decontamination methods for ISFs

115. There is no specific information relating to the environmental stability or methods of decontamination relating to ISFa and ISFb. ISFa and ISFb would be expected to have similar physical characteristics to other flaviviruses, which do not survive for extended periods outside the host or vector organism. The dengue virus RNA genome has been found to be stable in dried blood for up to nine days at room temperature (Public Health Agency of Canada, 2011). In contrast, Yellow fever virus particles are more fragile, with 0.16% or less of the virus remaining viable after 60 minutes when aerosolised at 27°C with a relative humidity of 30-80% (Mayhew et al., 1968). Flavivviruses like WNV have been found to be rapidly inactivated outside of a host, even when in an ideal isotonic environment (e.g. cell culture medium), with 90% of virus viability lost in within 24 hours and 99% lost after 72 hours at ambient temperature (Mayo and Beckwith, 2002).

116. Flaviviruses can be physically inactivated by ultraviolet light, desiccation, gamma-irradiation or heat. For example, Yellow fever virus or WNV are inactivated after 30 minutes at 60°C (Public Health Agency of Canada, 2010a, b). Dengue virus is sensitive to moist heat (121°C for at least 15 min), dry heat (160-170°C for at least 1 hour), and low temperature sterilization (ethylene oxide or plasma sterilization) or low PH (Public Health Agency of Canada, 2011).

117. Flaviviruses, are also sensitive to household disinfectants and detergents. For example, dengue virus is susceptible to 1% sodium hypochlorite, 2% gluteraldehyde, 2% peracetic acid, 70 % ethanol, iodophors, phenolic compounds, and 3-6% hydrogen peroxide (Public Health Agency of Canada, 2011).

Section 5 The GMO – nature and effect of genetic modifications

5.1 The genetic modification[3]

118. *Kunjin virus* (KUNV); family Flaviviridae, genus Flavivirus, is a member of the WNV group of flaviviruses.

[3] The identity of the parent organisms, as well as details of the design, construction and genetic modifications of the GMO, including the identity and function of the introduced Kunjin virus genes are under consideration as Confidential Commercial Information (CCI) under section 185 of the Act. Any confidential information will be made available to the prescribed experts and agencies.

119. KUNV has traditionally been associated with mild and rare disease in humans and horses in Australia (Prow, 2013). However, KUNV has caused rare outbreaks of severe neurological disease in horses in Australia, which appear to have been caused by a more virulent isolate of the virus referred to as $KUNV_{NSW2011}$ (Frost et al., 2013). KUNV is endemic to northern Australia and infections are usually asymptomatic (Hayes et al., 2005).

120. Birds serve as the major natural reservoirs for WNV and KUNV. KUNV is transmitted by the bite of a mosquito that has been infected by feeding on infected birds. Humans and most other mammals are regarded as dead-end hosts, since they do not produce sufficient viremia to infect mosquitoes (Prow, 2013).

121. An Australian isolate of KUNV ($KUNV_{MRM61C}$) was the source of the genes introduced into the GMOs. $KUNV_{MRM61C}$ was isolated from *Culex annulirostris* mosquitoes in Queensland in 1960 and has been demonstrated to be a naturally occurring attenuated strain of *Kunjin virus* (Prow et al., 2016) .

122. To generate the GMOs, a single copy of two genes that code for virion proteins of KUNV were incorporated into ISFa and ISFb in place of the corresponding genes of the parental viruses. The genetic modification results in the GMOs (ISFa-KUN and ISFb-KUN), which are live GM viruses that are intended to stimulate an immune response in vaccinated crocodiles to protect against infection by KUNV.

123. The GM viruses contain genes and regions of the genome[4] that together form the viral replicative complex from the ISF parental viruses. As a result it is expected that the GMOs will only replicate in insect cells and not in vertebrate cells, due to the incompatibility of the ISF replicative complex of the GMOs with vertebrate cell factors (Junglen et al., 2017).

5.1.1 *Characterisation of the phenotype of the GMOs*

124. The GMOs have been examined for the ability to replicate in vertebrate cells. The ISFa-KUN or ISFb-KUN GMOs were inoculated into mosquito cells (C6/36) and a range of vertebrate cell lines including mouse cells (mouse embryonic fibroblasts), monkey cells (Veros), hamster cells (BHK/BSR), chicken fibroblast cells (DF1) and crocodile cells (3CPL). An immunofluorescent antibody to the GMOs was used to test for the production of viral protein and infectious titre assays were used to detect production of infectious virus in the various cells examined. The GMOs were found to replicate in the C6/36 mosquito cell line, but not in any of the vertebrate cells tested[5].

125. No insect or vertebrate (animal or human) transmissibility studies of the ISFa-KUN and ISFb-KUN GMOs were provided in the application.

5.1.2 *Genotype stability and molecular characterisation of the GMOs*

126. The genetic stability of the ISFa-KUN and ISFb-KUN GMOs were examined by nucleotide sequence analysis (Deoxyribonucleic acid (DNA) sequencing) after serial passaging in mosquito cells. The DNA sequencing of the GMOs indicated no changes to the predicted amino acid sequences relative to those of the donor (KUNV-derived) or the flanking parental (ISFa- or ISFb-derived) proteins.

[4] Further information on the design, construction and genetic modifications of the GMO, including the identity and function of the introduced Kunjin virus genes replication properties of the ISFa-KUN or ISFb-KUN GMOs is presented in the CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

[5] Further information on the replication properties of the ISFa-KUN or ISFb-KUN GMOs is presented in the CCI Attachment to the RARMP, which is available to the prescribed experts and agencies that are consulted on the RARMP.

Section 6 Receiving environment

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

6.1 Site of release

128. The GMOs will be trialled at two crocodile farms in the Litchfield Council in the Northern Territory: Darwin Crocodile Farm, Bees Creek; and Janamba Crocodile Farm, Middle Point.

6.2 Related viral species in the receiving environment

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

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

131. The major mosquito-vectored flaviviruses in the Australian environment, including the Northern Territory, are *Murray Valley encephalitis virus*, the *Kunjin virus* and *Japanese encephalitis virus*. Other flaviviruses present in Australia include *Alfuy virus*, *Kokobera virus* (van den Hurk et al., 2001), *Hepatitis C virus,* and seasonal outbreaks of *Dengue virus* (Liu et al., 2005; 2006; 2008; Fitzsimmons et al., 2009).

132. The parent insect-specific flaviviruses were isolated from mosquitoes in northern Australia, so are likely to be in the release area. Recent studies have identified ISFs in various regions of Australia, however the majority of these are poorly characterised (Blitvich and Firth, 2015; Colmant et al., 2017).

6.3 Similar genetic material in the environment

133. The parent organisms are normally associated with mosquitoes in northern Australia, indicating that the parental virus genetic material is already associated with the region (northern Australia) where the field trials are to take place.

134. The genes introduced into the ISF parental viruses were derived from a naturally occurring Australian isolate of *Kunjin virus*, therefore similar genetic material would already be present in the environment.

6.4 Potential hosts in the environment

135. The parent organisms for the GMOs are normally associated with mosquitoes in northern Australia. Therefore mosquitoes, and possibly other dipterans, found in northern Australia are potential hosts for the GMOs.

136. The GMOs are able to replicate in insect cells but not in vertebrate cells, indicating that crocodiles, other animals and humans are not potential hosts.

Section 7 Previous authorisations

7.1 Australian authorisations

137. The GMOs have never been registered in Australia or elsewhere.

138. Work to develop the GMO in the laboratory including testing and preliminary experiments have been authorised under the Act as Notifiable Low Risk Dealings (NLRDs) conducted by The University of Queensland.

7.2 International authorisations and experience

139. No application for the use or marketing of the GMOs has been submitted to overseas regulatory authorities.

Chapter 2 **Risk Assessment**

Section 1 Introduction

140. Risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs (Figure 4). Risks are identified within the established risk context (see Chapter 1) and take into account current scientific and technical knowledge. Uncertainty, and in particular knowledge gaps, is considered throughout the risk assessment process.

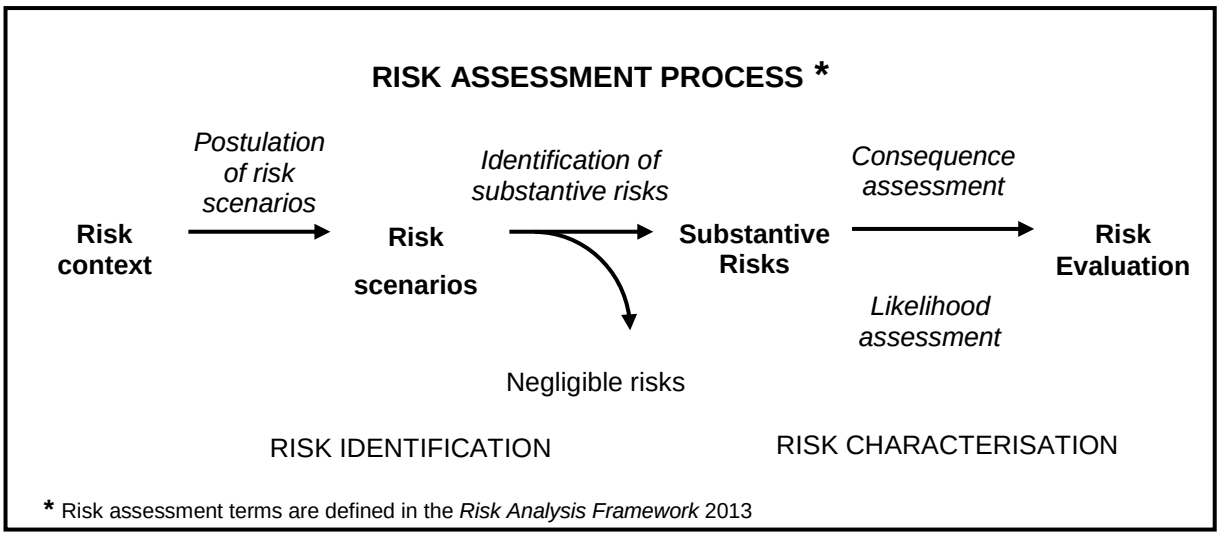

Figure 4. The risk assessment process

141. Risk identification first considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways whereby dealings with a GMO (risk scenarios) may, in the short and long term, harm people or the environment.

142. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. Substantive risks are further assessed when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

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

144. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. Risk evaluation then combines the Consequence and Likelihood assessments to determine level of risk and whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk Identification

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

i. Source of potential harm (risk source)

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

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

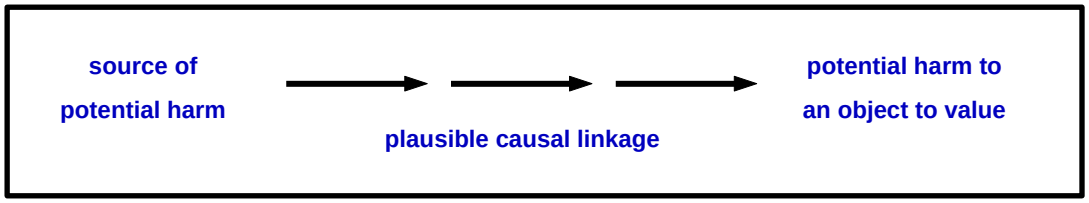

Figure 5. Components of a risk scenario

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

- the proposed dealings, which are conduct experiments with the GMO, transport and disposal of the GMO, and possession (including storage), supply and use in the course of any of these dealings
- restrictions placed on conduct of the experiments with the GMO, transport and disposal of GMO by other regulatory agencies, the relevant Territory and local councils
- characteristics of the parent organism
- routes of exposure to the GMO
- potential for transmission
- potential exposure to the same genes from environmental sources
- the release environment and
- practices during and after administration of the GMOs including crocodile farming practices.

147. Flaviviruses are normally transmitted horizontally between vertebrate hosts and an arthropod host or through direct blood contact. Aerosol transmission is not considered as a viable route of infection for flaviviruses, including the GMOs. Therefore aerosol transmission will not be considered further.

148. People working at or visiting the trial sites (crocodile pens) could be exposed to GMOs if they were shed from the vaccinated crocodiles. This could occur when handling the GMO-treated crocodiles (alive or dead carcasses), feeding the animals, cleaning pens and equipment used on site, and handling of waste. However flaviviruses are not known to be shed from infected hosts, so the only likely shedding of GMOs is from the injection site immediately after inoculation. As discussed in Chapter 1, Paragraph 114, flaviviruses are highly susceptible to degradation outside of the host organism and are rapidly degraded even under ideal conditions. If shedding was to occur, the GMOs would be expected to deteriorate quickly in field trial conditions. In addition, the GMOs are replication defective in vertebrate cells (e.g. Human and animal cells), therefore any exposure to people or animals would not result in a viral infection. For these reasons, transmission of the GMOs from crocodiles through shedding and subsequent inadvertent contact with people or animals will not be considered further.

149. After inoculated crocodiles reach the appropriate age for harvesting (12-30 months after inoculation), they would be processed on-site in the same way as for other commercial crocodiles at the farm sites. The GMOs are replication defective in vertebrate cells and the GMO viral particles are expected to be rapidly cleared by the crocodiles immune response. Any processing of inoculated crocodiles for meat or other products would not occur until months after inoculation, when no GMO would be present. Any inoculated crocodiles that die soon after inoculation will be disposed of according to normal farm protocols of as biohazardous waste. The applicant has proposed to test for the persistence of the GMOs in inoculated crocodiles on the farms. Animals thought to contain viable

GMOs will not be processed for meat or animal products, but will be euthanised and disposed of as biohazardous waste. For these reasons, exposure or transmission of the GMOs from crocodiles to people or animals via food or other products of inoculated crocodiles will not be considered further.

150. The GMOs and samples containing the GMO are proposed to be transported and stored according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols for the handling of GMOs to minimise exposure to the GMOs. The GMOs proposed for field trial in this application could be transported according to these guidelines under an NLRD authorisation, so risks associated with such transport will not be not be further assessed.

2.1 Postulated risk scenarios

151. Three risk scenarios were postulated, as summarised in Table 1. These risk scenarios were evaluated considering both short and long term effects, and in the context of practices proposed by the applicant. Detailed evaluations of these scenarios are provided later in this section. None of the risk scenarios were identified as a risk that could be greater than negligible and warranting further scrutiny.

Table 1 Summary of risk scenarios from dealings with GMO

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
1.	GM ISFa-KUN or ISFb-KUN	i. Exposure of people undertaking dealings to GMO via needle stick/sharps injury during GMO preparation, injection or sample collection ⬇ ii. Transduction of cells ⬇ iii. Expression of GM vaccine proteins ⬇ iv. Inappropriate immune response and/or establishment of viral infection	Ill health, increased disease burden	No	– Animal handling and containment procedures for crocodile farms follow standardised biosecurity measures – PPE (e.g. gloves) minimises potential exposure – Only trained and experienced personnel will prepare and administer GMOs and will be trained in use and disposal of sharps – Only trained and experienced personnel will handle animals, including during and after vaccination – Animals will be restrained during injection or sampling – Any dose of GMO received through accidental exposure would be far smaller than that administered during inoculation – GMOs are replication defective in vertebrate cells (e.g. human cells) and viral particles are expected to be rapidly cleared by the immune response – Any expression of the introduced KUNV proteins will be transient
2.	GM ISFa-KUN or ISFb-KUN	i. Crocodiles inoculated with GMOs ⬇ ii. Mosquitos or other insects pick up the GMO via horizontal transmission (e.g. feeding/blood meal) ⬇ iii. GMOs infect and replicate in mosquitos or other insects	Increased disease burden for mosquito or other insects, impacts on insect biodiversity	No	– GMO is administered subcutaneously or intramuscularly and is expected to remain localised at the site of injection in the crocodile – As the GMOs are replication defective in vertebrate cells, the crocodiles immune system would be expected to clear the GMOs within hours to a few days of inoculation – For a limited time, only a small portion of GMO would be present in the crocodile vascular system to be available for transmission to a mosquito or insect via blood meal – The parental ISFs are natural commensals of mosquitoes in Northern Australia and the genetic modifications are not expected to alter the non-pathogenic nature of this relationship

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
					– Due to the transmission characteristics of ISFs, any insect infected with the GMO would only be expected to transmit the GMO to its own progeny and not to the wider insect population
3.	GM ISFa-KUN or ISFb-KUN	i. Crocodiles inoculated with GMOs ⬇ ii. Mosquitos or other insects pick up the GMOs via horizontal transmission (e.g. feeding/blood meal) ⬇ iii. Mosquito or insect is already, or later becomes, infected with another flavivirus ⬇ iv. Both viruses infect and replicate in the same cell ⬇ v. GM virus recombines with other flavivirus virus in the host ⬇ vi. Recombinant virus establishes infection ⬇ vii. Mosquito or insect spreads recombinant virus to animals or people ⬇ viii. Recombinant virus infects and replicates animals or people	Increased disease burden for animals or people	No	– GMO is administered subcutaneously or intramuscularly and is expected to remain localised at the site of injection in the crocodile – For recombination to occur, the GMO and other flavivirus need to be present in the same cell – Recombination between flaviviruses is extremely rare and typically results in viral attenuation – All of the genetic material in the GMOs is present in the environment in northern Australia, and therefore already opportunity for recombination in the absence of the GMO.

2.1.1 *Risk scenario 1 – Exposure of people undertaking dealings to GMO*

Risk source	GM ISFa-KUN or ISFb-KUN
Causal pathway	i. Exposure of people undertaking dealings to GMO via needle stick/sharps injury during GMO preparation, injection or sample collection ⬇ ii. Transduction of cells ⬇ iii. Expression of GM vaccine proteins ⬇ iv. Inappropriate immune response and/or establishment of viral infection
Potential harm	Ill health, increased disease burden

Risk source

152. The source of potential harm for this postulated risk scenario is GM ISFa-KUN or ISFb-KUN.

Causal Pathway

153. There are a number of ways that people may be exposed to the GMOs while undertaking the dealings as part of this field trial.

154. Exposure to people involved in the field trials may occur via needle stick/sharps injury during GMO preparation, injection or sample collection. The frozen GMOs need to be thawed and reconstituted with buffer containing adjuvant. The applicant proposes that workers preparing the GMOs or cleaning spills would wear personal protective equipment (PPE), including gloves.

155. Prior to inoculation, people conducting the dealings would be trained in handling, preparing and administering the GMOs. Animals will be restrained by a trained crocodile farm handler wearing puncture-resistant gloves. Inoculation and the collecting of samples would be conducted by appropriately trained farm/research personnel or a registered veterinarian, wearing gloves, safety glasses and a puncture-resistant glove on the hand not holding the syringe. Access to crocodile pens and handling of crocodiles is restricted to authorised personnel wearing appropriate PPE.

156. Following administration of the GMOs, used vials and other waste generated during the vaccination procedure and any unused GMOs, will be subject to decontamination using 10% bleach, 70% ethanol or disposal as biohazardous material. After handling the GMOs, any work surfaces used will be decontaminated with an appropriate chemical disinfectant, following standard farm procedures.

157. As described in Chapter 1 Section 4.2, the parent organisms are not able to replicate in vertebrates (including people); the genetic modifications are not expected to change this characteristic; and the applicant has shown that the GMOs do not replicate in vertebrate cells. Therefore any exposure via needle-stick or sharps injury to persons conducting the dealings would not result in viral infection.

Potential harm

158. The GMOs cannot replicate in vertebrate cells, therefore exposure to the GMOs will not lead to viral infection/disease in humans.

159. As the parent ISF's and KUNV are present in the environment, people would already be exposed to the expressed proteins. As the GMOs cannot replicate in vertebrate cells, any exposure to the GMOs and the viral proteins would be at low levels and transient, minimising the potential for an inappropriate immune response.

Conclusion

160. Risk scenario 1 is not identified as a substantive risk because exposure is limited by the proposed practices, and the GMO is not expected to cause disease or other harms in people who are exposed. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.2 *Risk scenario 2 – Exposure of mosquitos or other insects to the GMOs*

Risk source	GM ISFa-KUN or ISFb-KUN
Causal pathway	i. Crocodiles inoculated with GMOs ⬇ ii. Mosquitos or other insects pick up the GMO via horizontal transmission (e.g. feeding/blood meal) ⬇ iii. GMOs infect and replicate in mosquitos or other insects
Potential harm	Increased disease burden for mosquito or other insects, impacts on insect biodiversity

Risk source

161. The source of potential harm for this postulated risk scenario is GM ISFa-KUN or ISFb-KUN.

Causal Pathway

162. Mosquitos or other insects may be exposed to the GMO at the trial site when feeding on inoculated crocodiles or when in contact with meat or animal products from inoculated crocodiles. While ISFs have been isolated exclusively from mosquitoes, there is a possibility that ISFs may be found in other dipteran insects (see paragraph 104).

163. As discussed in Risk Scenario 1, the GMOs are replication defective in vertebrate cells and are not expected to persist in the inoculated crocodile.

164. Additionally, after the GMO is administered subcutaneously or intramuscularly, it is expected to be taken by up antigen presenting cells (macrophages and dendritic cells) in the region of the inoculation and sequestered into the local lymph nodes where it will be broken down by the immune system. There is potential for some of the GMOs to enter the crocodile's vasculature system, and therefore to be available to feeding insects, however the amount would be minimal. As horizontal transmission of flaviviruses from a vertebrate host to an insect host normally requires a high level of viremia, and the level of GMO in the blood of inoculated crocodiles in expected to be minimal, transmission form an inoculated crocodile to a feeding insect is not considered plausible.

165. Any processing of inoculated crocodiles for meat or other products would not occur until months after inoculation, when no GMO would be present. Any inoculated crocodiles that die soon after inoculation will be disposed of according to normal farm protocols by a waste contractor.

166. The applicant has proposed to test for the persistence of the GMOs in inoculated crocodiles on the farms. Animals thought to contain viable GMOs will not be processed for meat or animal products, but will be euthanised and disposed of as biohazardous waste according to Commonwealth and Territory requirements (Chapter 1 Section 3.3.9).

167. The applicant has also proposed to monitor for the presence of the GMOs in non-inoculated crocodiles and mosquitos on the farms. If the GMOs are found to be transmitted to other animals or insects, the applicant will cease GMO-inoculations and implement measures to contain the GMO and prevent further dispersal or persistence of the GMO in the environment.

Potential harm

168. The parental ISFs are natural commensals of mosquitoes in northern Australia, and the genetic modifications are not expected to alter the non-pathogenic nature of this relationship. As discussed in Chapter 1 Section 4.2.1, many arbovirus flaviviruses, including KUNV, are transmitted via insect hosts and do not appear to have significant detrimental effects on insect persistence in the environment. There are reports that WNV (arbovirus) infections in mosquito vectors can be cytopathic, however there is no definitive evidence that WNV infections result in significant changes in survival between infected and uninfected mosquitoes.

169. The primary means of transmission of ISFs is vertically from mother to progeny, with sexual transmission potentially playing a minor role. (Bolling et al., 2012). This would limit spread of the GMO and minimise any potential impact on mosquito populations.

Conclusion

170. Risk scenario 2 is not identified as a substantive risk because exposure is minimised by the route of inoculation, the inability of the GMOs to replicate in the crocodiles, the GMOs are not expected to cause disease in mosquitos or other insects that may be exposed, and there is little potential for spread of the GMOs in insect populations. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.3 *Risk scenario 3 – Horizontal transfer of genes or genetic elements*

Risk source	GM ISFa-KUN or ISFb-KUN
Causal pathway	i. Crocodiles inoculated with GMOs ⬇ ii. Mosquitos or other insects pick up the GMOs via horizontal transmission (e.g. feeding/blood meal) ⬇ iii. Mosquito or insect is already, or later becomes, infected with another flavivirus ⬇ iv. Both viruses infect and replicate in the same cell ⬇ v. GM virus recombines with other flavivirus virus in the host ⬇ vi. Recombinant virus establishes infection ⬇ vii. Mosquito or insect spreads recombinant virus to animals or people ⬇ viii. Recombinant virus infects and replicates animals or people
Potential harm	Increased disease burden for animals or people

Risk source

171. The source of potential harm for this postulated risk scenario is GM ISFa-KUN or ISFb-KUN.

Causal Pathway

172. Mosquitos and other insects are commonly exposed to flaviviruses. For example KUNV is endemic in northern Australia, including around the trial sites, and there are occasional outbreaks of other flaviviruses in the region. Insects may be infected by more than one flavivirus.

173. Mosquitos or other insects may be exposed to the GMO at the trial site when feeding on inoculated crocodiles or when in contact with meat or animal products from inoculated crocodiles, however as described in risk scenario 2 this is unlikely.

174. Even if a mosquito or other insect were to be infected by the GMO and another flavivirus, the frequency of recombination in flaviviruses is extremely low (Taucher et al. 2010).

Potential harm

175. Recombination between the GMO and another flavivirus strain could result in viral progeny having any permutation of genomic segments of the two parent strains. In theory, recombination could produce a less, similar or more virulent phenotype than either parent strain. However, artificially produced recombinants between a number of flaviviruses have all shown reduced pathogenesis and/or virulence when compared with the parent viruses (for example: Pletnev et al., 1992; Arroyo et al., 2001; Pletnev et al., 2002; Mathenge et al., 2004; Guy et al., 2008; Domingo and Niedrig, 2009). Therefore it is not expected that recombination between the GMO and a circulating flavivirus strain would lead to virus which is more pathogenic or virulent than the circulating flavivirus. Additionally, given that the GMOs are not able to replicate in vertebrate cells, recombination with a dual-host flavivirus is not likely to generate a new virus which replicates efficiently in vertebrates, which would limit its potential spread and persistence.

176. Baseline information on the presence of the parental viruses and introduced genes or similar genetic elements is provided in Chapter 1. The parental viruses and introduced genetic elements are derived from naturally occurring viruses already present in the environment in northern Australia. Therefore all of the genetic material is already available for recombination.

Conclusion

177. Risk Scenario 3 is not identified as a substantive risk as exposure of mosquitos to the GMOs is unlikely (as described in risk scenario 2), recombination among flaviviruses is rare, and any recombinant flavivirus strain is likely to be of less or similar virulence than the parental viruses. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

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

- uncertainty about facts:

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

  - 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.

180. 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.

181. As field trials of GMOs are designed to gather data, there are generally data gaps when assessing the risks of a field trial application. However, field trial applications are required to be limited and controlled. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO, and thus decrease the likelihood of harm.

182. For DIR 159, uncertainty is noted in relation to the length of time it will take for an inoculated crocodile's immune system to clear the GMOs from its body. The route of inoculation and the inability of the GMO to replicate in crocodiles restrict the potential of the GMOs to be transmitted to mosquitos or other insects. The applicant has proposed to test for the presence of the GMOs in inoculated crocodiles, and to not release GMO-inoculated crocodiles into the general population or for processing unless testing confirms that that the GMOs have been cleared.

183. Some uncertainty is also noted in relation to the behaviour of the GMOs in mosquitos and other insects, including their ability for transmission, replication and their potential effects on infected insects. However, the opportunity for exposure of mosquitos and insects is minimal. Nevertheless, the applicant has proposed to test for the presence of the GMOs in non-inoculated crocodiles and mosquitos to address these areas of uncertainty. Data from this testing would be made available to the OGTR.

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

Section 4 Risk evaluation

185. 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.

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

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

187. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the proposed release sites, limits and controls proposed by the applicant, biosecurity measures, local council and state requirements, and considering both the short and long term consequences, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 1and include:

- the limited host range of the GMOs
- the transmission characteristics of the parent organisms
- limited ability and opportunity for the GMOs to transfer the introduced genes
- suitability of the controls proposed by the applicant.

188. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GMO into the environment are considered to be negligible. The Risk Analysis Framework, which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, 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[7].

[7] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. The Regulator has allowed 5 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 **Risk management plan**

Section 1 Background

189. 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 licence conditions.

190. 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.

191. 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.

192. 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

193. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed trial of the GM viruses. These risk scenarios were considered in the context of the scale of the proposed release and the proposed containment measures (which include standard industry practice, and state and local requirements), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

194. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been drafted to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMO and its genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in Chapter 4 (the draft licence).

3.1 Draft licence conditions to limit and control the release

3.1.1 *Consideration of limits and controls proposed by The University of Queensland*

195. Chapter 1 provide details of the limits and controls proposed by The University of Queensland in their application. Many of these are discussed in the three risk scenarios characterised for the proposed release in Chapter 2. The appropriateness of these controls is considered further below.

196. The applicant proposes that the field trials are carried out at 2 crocodile farms in the Northern Territory and the duration of the field trials would be limited to five years. The applicant proposes

inoculating up to 2800 crocodiles. Access to the farms participating in the trial would be controlled. These measures would minimise the potential exposure of people and other organisms to the GMO, and are included in the draft licence.

197. The GMOs will be administered to juvenile crocodiles (between 3 to 6 month's old, weighing approximately 350 grams) by subcutaneous or intramuscular injection into the tail. The GMOs would only be administered by a suitably trained farm or research personnel, or registered veterinarian associated with the trial. The applicant proposes that workers preparing the GMOs would wear PPE (Risk scenario 1). During GMO administration or sampling, animals will be restrained by a trained crocodile farm handler wearing puncture-resistant gloves. The persons performing the injections or taking blood samples will wear a puncture-resistant glove on the hand not holding the syringe. Wearing personal protective equipment, including gloves would minimise exposure of workers to the GMOs and are included in the licence. These measures have been included in the draft licence. Conditions are also included in the draft licence requiring that experimentation with the GMOs or GMO-treated crocodiles to be undertaken within a nominated trial area.

198. As discussed in Chapter 1, the GMOs are expected to be broken down by the crocodile's immune response and cleared from the crocodile within hours to a few days of vaccination, however there is no empirical evidence to confirm this expectation. The applicant has proposed to test crocodiles for the presence of the GMOs at various time-points post-inoculation. Inoculated crocodiles would not be released to the general crocodile population if tests indicate that they are likely to contain the GMO. Each GMO-inoculated crocodile will be tagged for identification. To minimise the exposure of people or insects to the GMO (Risk scenarios 1, 2 and 3), a condition has been included in the draft licence which prohibits the release of crocodiles thought to contain viable GMOs to the general crocodile population or for processing. Procedures to account for all GMO-inoculated crocodiles, including tagging and record-keeping, must be in place. Non-inoculated crocodiles must not be housed in the same pens GMO-inoculated crocodiles.

199. As noted in Chapter 1, a range of biosecurity measures and general farm management procedures are routinely applied at crocodile farms to minimise pathogen occurrence and spread. Decontamination measures for people, sheds and farm equipment are proposed by the applicant. Most of these standard measures were not considered important in the risk assessment due to mechanism of transmission, limited host range and inability of the GMOs to replicate in vertebrate cells, as well as the limited persistence of flaviviruses in the environment outside a host cell. Therefore these general measures are not included in draft licence conditions. However, appropriate waste management was considered in relation to Risk scenario 1. Animal carcasses and other biological waste considered to contain viable GMOs will be disposed of in secured bins for disposal by a waste contractor. All equipment and materials contaminated with the GMO such as bottles, vials, needles and other GMO-contaminated materials would be disinfected after use or disposed of as biohazardous material by a waste contractor. Conditions are included in the draft licence requiring that all waste and equipment that may be contaminated with the GMO is to be decontaminated or disposed of as biohazardous material, in accordance with applicable legislation.

200. The GMOs and samples containing the GMOs would be transported and stored according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols for the handling of GMOs to minimise exposure to the GMOs. The draft licence requires that transport and storage of the GMOs and samples be in accordance with the Guidelines.

3.1.2 *Summary of draft licence conditions to be implemented to limit and control the release*

201. A number of licence conditions have been drafted to limit and control the proposed release, based on the above considerations. These include requirements to:

- limit the field trials to inoculation of up to 2,800 crocodiles at the 2 nominated farms in the Northern Territory, from the date of licence issue to May 2023
- restrict access to trial areas

- ensure personnel involved in the trial are appropriately trained
- test crocodiles for the presence of the GMOs post-inoculation, and segregate GMO-inoculated crocodiles from other crocodiles until the GMOs are determined to be no longer present
- ensure compliance with Northern Territory requirements for waste disposal
- transport and store the GMO and samples from GMO-treated crocodiles in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, in force at the time.

3.2 Other risk management considerations

202. 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
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 *Applicant suitability*

203. 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.

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

205. In addition, the applicant organisation must have access to a properly IBC and be an accredited organisation under the Act.

3.2.2 *Contingency Plan*

206. If a licence were issued, The University of Queensland would be required to submit a contingency plan to the Regulator before dealing with the GMOs. This plan would detail measures to be undertaken in the event of unexpected persistence of the GMO in inoculated crocodiles or transmission of the GMO to animals or insects other than inoculated crocodiles.

207. The University of Queensland would also be required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modification in a recipient organism. This methodology would be required before dealing with the GMO.

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

208. If a licence were issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to dealing with the GMO, The University of Queensland would be required to provide a list of people and organisations that would be covered by the licence, or the function or position where names are not known at the time.

3.2.4 *Reporting requirements*

209. If issued, the licence would require 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.

210. A number of written notices would also be required under the licence regarding dealings with the GMO at each farm, to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices would include:

- description/diagram/map of the farm and any associated sheds, ranges, houses or buildings (if relevant) in the trial area and what each is used for
- expected date of inoculation with the GMO
- number and age of crocodiles to be inoculated with the GMOs
- identification of the particular hatchling facility or pens where the GMO-inoculated crocodiles will be kept.

3.2.5 *Monitoring for Compliance*

211. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

212. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

213. 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 Issues to be addressed for future releases

214. Additional information has been identified that may be required to assess an application for a commercial release of the GMO, or to justify a reduction in limits and controls. This includes:

- Information obtained from testing for persistence of the GMOs in inoculated crocodiles and for presence of the GMOs in other crocodiles or in mosquitos at the trial sites.

Section 5 Conclusions of the consultation RARMP

215. The risk assessment concludes that the proposed limited and controlled release of the GMO poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

216. If a licence were issued, conditions would be imposed to limit the release to the proposed scale, location and duration, and to restrict the spread and persistence of the GMO and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 **Draft licence conditions**

Section 1 Interpretations and Definitions

1. In this licence:

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

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

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

   (d) words importing persons include a partnership and a body whether corporate or otherwise;

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

   (f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

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

2. In this licence:

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

'**Annual Report**' means a written report provided to the Regulator by 30 September of each year containing all the information required by this licence to be provided in the Annual Report for the preceding financial year.

'**APVMA**' means the Australian Pesticides and Veterinary Medicines Authority.

'**Crocodile'** means Salt water crocodile (*Crocodylus porosus*) raised or grown for meat or animal products.

'Decontaminate' (or **'Decontamination'**) means, as the case requires, kill the **GMO** by one or more of the following methods:

a) chemical treatment;

b) autoclaving;

c) high-temperature incineration;

d) deep burial; and

e) a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.

'**Equipment**' includes, but is not limited to vials, needles, swabs, clothing, gloves, cleaning equipment and tools.

'**GM**' means genetically modified.

'**GMO**' means the genetically modified organisms that are the subject of the dealings authorised by this licence.

'GMO stock' means the undiluted GMOs as supplied to crocodile farms from the University of Queensland.

'**OGTR**' means the Office of the Gene Technology Regulator.

'**Participating farm**' means a crocodile farm on which a Trial area exists.

'Pen' means a building, or enclosure within a building, with concrete floor, walls and enclosed roof that are sufficient to physically separate and contain the crocodiles.

'**Personal information**' means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

(a) whether the information is true or not; and

(b) whether the information is recorded in a material form or not.

'**Regulator**' means the Gene Technology Regulator.

'**Sample**' means any biological material collected for analysis from a crocodile, mosquito or other source at a Participating farm, and which may reasonably be expected to contain GMOs.

'**Trial area'** means an area within a Participating farm where the GMO is stored, prepared or used as part of the trial. This includes, but is not limited to, the following:

(a) Pens where crocodiles are inoculated with the GMO and subsequently housed;

(b) areas where Samples are taken from, or autopsies conducted on, GMO-inoculated crocodiles;

(c) areas used to prepare the GMO for inoculation; and

(d) storage areas for the GMO stock, carcasses and waste that may potentially be contaminated with the GMO.

Section 2 General conditions and obligations

3. The holder of this licence ('the licence holder') is the University of Queensland.

4. The GMO covered by this licence is GM live insect-specific flavivirus, as described in **Attachment A** of the licence.

Note: Attachment A is not included in the draft licence as the GMOs are described in this Risk Assessment and Risk Management Plan.

5. The dealings authorised by this licence are to:

a) conduct experiments with the GMO;

b) transport of the GMO;

c) disposal of the GMO;

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

6. This licence does not authorise dealings with the GMO that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

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

8. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by this licence as part of the field trial, and (to the extent that the GMO may be present at the time) persons who subsequently transport or handle waste containing GMOs.

9. The licence holder must keep a record of all persons covered by this licence who are engaged in the field trial on a Participating farm (including for transport to or from a Participating farm), and must keep a record of the contact details of the project supervisor(s) for the licence.

Note: Where contractors are used to conduct transport or decontamination/disposal, it is sufficient to record the company name and the position or job title of the person(s) conducting the dealing.

Obligations of the Licence Holder

10. The licence holder must notify the Regulator in writing as soon as practically possible if any of the contact details of the contact person(s) for the licence or project supervisor(s) change from that notified in the licence application or subsequently.

Note: Please address correspondence to ogtr.applications@health.gov.au

11. The licence holder must notify the Regulator in writing, and supply a copy, of any permit issued by the APVMA related to the dealings covered under this licence, within 14 days of the permit being issued or any change to permit conditions being made.

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

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

13. The licence holder must:

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

i) any relevant conviction of the licence holder occurring after the issue of this licence; and

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

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

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

14. The licence holder must be able to access and control all Trial areas to the extent necessary to ensure compliance with conditions of this licence for the duration of the life of the licence.

The following conditions seek to ensure that persons conducting the dealings covered by licence conditions are aware of the licence conditions and appropriate processes are in place to inform people of their obligations.

15. Prior to conducting any dealings with the GMO, the licence holder must provide to the Regulator (for each Participating farm, where relevant) the following information:

(a) names of all organisations and persons, or functions or positions of the persons, who will be engaged in the dealings covered by the licence, with a description of their responsibilities;

Note: Examples of functions or positions are 'project supervisor', 'farm manager', 'farm labourer' etc.

(b) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them;

Note: This may include a description of any contracts, training, labelling, contractual agreements with other organisations or persons such as a crocodile farm owner(s), commercial waste providers or courier companies.

(c) how the licence holder will access and control Trial areas to the extent necessary to ensure compliance with conditions of this licence for the duration of the licence.

Note: This may include a description of any contracts, agreements, or other enforceable arrangements.

(d) written methodology to reliably detect the GMO, or the presence of the genetic modification in a recipient organism.

16. Any changes to the information provided under Condition 15 must be communicated in writing to the Regulator within 14 days of the changes occurring.

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

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

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

(c) the surrender of the licence.

18. The licence holder must not permit a person covered by this licence to conduct any dealing unless:

(a) the person has been informed of any particular licence conditions that apply to them, including any variation of them; and

(b) the licence holder has obtained from the person a signed and dated statement that the person:

i) has been informed of the particular licence condition(s) including any variation of them; and

ii) has understood and agreed to be bound by the licence conditions, or variation.

19. The licence holder must ensure that all persons undertaking dealings at the Trial areas (e.g. handling the GMO, GMO-inoculated crocodiles, or any Equipment or waste potentially contaminated with GMO) are trained in handling and decontamination of the GMOs as documented in the Compliance Management Plan provided under Condition 43.

20. The licence holder must:

(a) inform the persons covered by this licence to whom a particular condition applies that any personal information relevant to the administration and/or enforcement of the licence may be released to the Regulator; and

(b) provide the Regulator, if requested, with copies of the signed and dated statements referred to in Condition 18.

Provision of new information to the Regulator

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

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

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

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

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

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

(a) the licence holder will be taken to have become aware of additional information of a kind mentioned in paragraph 21(a) if he or she was reckless as to whether such information existed; and

(b) the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in paragraph 21(b) or 21(c), if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.

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

22. If the licence holder is required to inform the Regulator under the immediately preceding condition, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of the incident via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours. Notification without delay will allow the OGTR to conduct a risk assessment on the incident and attend the location if required.

23. If the licence holder informs the Regulator under the immediately preceding condition and the Regulator requests further information, such information must be provided in a manner, and within the time period, stipulated by the Regulator.

Obligations of persons covered by the licence

24. Persons covered by this licence engaged in the field trial must not deal with the GMO except as expressly permitted by this licence.

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

Section 3 Limits and control measures

Note: This licence does not expressly authorise or prohibit any dealings or storage in facilities certified by the Regulator. Under the Act it is not an offence to deal with a GMO if the dealing is otherwise licenced or if it is a notifiable low risk dealing (NLRD) or an exempt dealing and complies with all relevant statutory requirements.

Limits on the release

The following licence conditions maintain the risk assessment context within which the application was assessed, by imposing limits on where and when the GMO may be released, and on other activities that can be undertaken.

26. Inoculation and housing of GMO-inoculated crocodiles may only occur at Darwin Crocodile Farm and Janamba Crocodile Farm in the local government area of Litchfield Council, Northern Territory.

27. Only juvenile crocodiles, up to 9 months of age, may be inoculated with the GMOs.

28. A cumulative maximum of 2,800 crocodiles over the life of the licence may be inoculated with the GMOs.

29. Inoculation of crocodiles and storage of GMO stock must be completed by May 2023.

30. If experimentation and analysis with the GMOs, GMO-inoculated crocodiles or Samples, is not conducted in accordance with NLRD requirements, such activities may only be undertaken within a Trial area.

Controls on the release

The following licence conditions maintain the risk assessment context within which the application was assessed by restricting spread and persistence of the GMO, and apply to dealings on Participating farms and transport to and from these farms.

Practices at Participating farms

31. The licence holder must ensure that a copy of the licence is available and readily accessible to persons conducting dealings at Trial area.

32. Access to Trial areas must be restricted to only persons authorised by the Licence holder.

33. Signs indicating the presence of the GMO must be displayed at all entrances to Trial areas.

34. Inoculation of crocodiles with the GMOs must only occur inside a Pen.

35. Persons preparing or administering the GMOs must be appropriately trained as detailed in the Compliance Management Plan.

36. Persons preparing or administering the GMOs must wear personal protective equipment, including gloves. Puncture-resistance gloves must be used when there is a risk of sharps injury during these procedures (as detailed in the Compliance Management Plan).

37. Crocodiles must be inoculated with the GMOs by subcutaneous or intramuscular injection into the tail.

38. At least 20% of each batch of GMO-inoculated crocodiles must be tested for the presence of the GMOs within 4 weeks of their final inoculation, and periodically thereafter (as specified in the Compliance Management Plan), until no GMO is detectable.

39. GMO-inoculated crocodiles must be segregated from all other crocodiles kept at the Participating farm, up to and including 4 weeks after the last GMO inoculation and until the testing required by condition 38 indicates that the GMO are no longer present.

40. Mosquitoes and non-inoculated crocodiles on the Participating farms must be sampled and monitored for the presence of GMOs over the period of the trial (as specified in the Compliance Management Plan).

41. The licence holder must ensure that only a person holding an *environment protection licence* or a *best practice licence* under the Waste Management and Pollution Control Act (Northern Territory; WMPC Act) to conduct activities specified in clause 2 and 3 of Part 2 of Schedule 2 WMPC Act are engaged for disposal of waste potentially contaminated with the GMOs outside a Trial area.

Note: Condition 41 does not impose licence conditions on persons engaged to conduct waste disposal, however the licence holder is responsible for ensuring only people who are appropriately authorised for waste disposal in the NT are permitted to conduct the disposal.

Transport and storage of GMO stock and Samples

42. Transport and storage of the GMO stock or Samples must be in accordance with requirements for Physical Containment level 1 GM micro-organisms of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* in force at the time of transport or storage.

Section 4 Reporting and Documentation

Compliance management and Contingency plans

43. At least 14 days prior to inoculating crocodiles with the GMO at each Participating farm, a written Compliance Management Plan must be submitted to the Regulator, detailing:

(a) procedures for the use of sharps, handling of the GMOs during preparation and administration, decontamination of the GMOs and waste disposal;

(b) personal protective equipment to be used when preparing the GMO for inoculation, administering the GMO and taking samples from GMO-inoculated crocodiles (including details of situations for which the use of puncture resistant gloves are appropriate);

(c) a plan for the testing for the presence of GMOs in GMO-inoculated crocodiles at each Participating farm, including criteria for selection of crocodiles, types of the samples to be collected, and timing of sample collection (as required by Condition 38);

(d) a plan for the testing for the presence of the GMOs in non-inoculated crocodiles and mosquitos at each Participating farm, including details of type of samples to be collected and, for each sample type, criteria for determining where, when and how many samples are to be collected.

44. At least 14 days prior to inoculating crocodiles with the GMO at each Participating farm, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of:

(a) the GMO being detected in GMO-inoculated crocodiles later than 4 weeks post-inoculation;

(b) a GMO being detected in a Sample from crocodile other than a GMO-inoculated crocodile or mosquito;

(c) a person receives a needle stick/sharps injury during GMO preparation, inoculation or sample collection from an inoculated crocodile;

(d) a spill or unintended release of GMO (i.e. during transport between Trial areas or during inoculation);

(e) escape or loss of a GMO-inoculated crocodile that is required to be segregated from non-inoculated crocodiles according to Condition 39 from its Pen.

45. The Contingency Plans must include details of procedures to:

(a) ensure the Regulator is notified as soon as reasonably possible after the licence holder becomes aware of the event;

(b) if GMOs are detected in GMO-inoculated crocodiles later than 4 weeks post inoculation or in non-inoculated crocodiles or mosquitos, implement measures to minimise further persistence and dispersal of the GMO in the environment;

(c) if exposure through needle stick/sharps injury is suspected or confirmed, provide appropriate medical attention to affected persons as necessary;

(d) In the event of a spill or unintended release of GMO, procedures to contain and decontaminate the GMO;

(e) in the event of an escape or loss of a GMO-inoculated crocodile that is required to be segregated from non-inoculated crocodiles according to Condition 39, procedures to locate the animal and return it to containment.

46. If any of the events described in Condition 43 occur, the appropriate procedure(s) from the Contingency Plan must be implemented.

Notices of commencement and completion of inoculations

47. At least 14 days prior to commencing dealings with the GMO at a Participating farm, the licence holder must provide the Regulator with a detailed diagram or map of the farm, including the proposed Trial areas, the Pens and any other buildings, and what each structure is used for.

48. The licence holder must notify the Regulator in writing at least 7 days before inoculation of each batch of crocodiles with the GMOs at each Participating farm, and must include the following details:

(a) expected dates of inoculation with the GMO;

(b) number and age of crocodiles to be inoculated with the GMO;

(c) identification of the particular Pens where the GMO-inoculated crocodiles will be kept.

Note: The notices required by conditions 47 and 48 may be combined for the first inoculation at a particular Participating farm, and a notice under condition 48 may cover more than one batch of crocodiles if the relevant details for each batch can be accurately provided.

49. Any changes to the details provided under Condition 50 must be provided to the Regulator within 7 days.

50. For each Participating Farm, the Licence Holder must notify the Regulator of the following:

(a) for the first batch of GMO-inoculated crocodiles, at least 14 days prior to the expected date, the intention to release the GMO-inoculate crocodiles into the general crocodile population, along with a summary of the testing data (as required under Conditions 38) supporting the release;

(b) for all other batches of GMO-inoculated crocodiles, their release into the general crocodile population within 7 days of the event. If there are no further GMO-inoculated crocodiles to be released, and no further inoculations to be conducted, this information must be included in the notification.

Annual Report

51. By 30 September each year, the licence holder must provide to the Regulator an Annual Report for the preceding financial year, including for the period:

(a) the number of crocodiles inoculated with the GMO at each Participating farm;

(b) the number of GMO-inoculated crocodiles released into the general crocodile population at each Participating farm; and

(c) a summary of the results of testing for the GMO in GMO-inoculated crocodiles, non-inoculated crocodiles and mosquitos (as required under Condition 38 and according to the Compliance Management Plan provided under Condition 43);

Records to be maintained

52. The following records must be made and kept for the life of this licence, and made available to the Regulator on request:

(a) measures taken to ensure that Pens, Trial areas and Participating farm fencing keep crocodiles securely enclosed, including inspection and maintenance activities, as applicable;

(b) details of each batch of crocodiles inoculated with the GMO as notified to the Regulator under Condition 48;

(c) details of each release of GMO-inoculated crocodiles to the general crocodile population;

(d) monitoring and testing data as required under Conditions 38 and 40, and according to the Compliance Management Plan provided under Condition 43.

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