

MRCU / Oxitec application for operational deployment of genetically altered *Aedes aegypti* (OX513A) throughout the Cayman Islands.

Department of Environment screening evaluation

The National Conservation Council has received an application for a permit to release a genetically altered organism, from the Mosquito Research and Control Unit (Cayman Islands Government) and Oxitec (Cayman) Ltd.

The applicants propose to commence operational deployment of OX513A *Aedes aegypti* male releases as a tool to suppress the invasive wild population of *Aedes aegypti*, which currently poses an arbovirus disease risk to the human population on Grand Cayman and Cayman Brac. The application is made under section 35(2) of the National Conservation Law 2013, which states “A person who wishes to introduce or release in any part of the Islands a live or viable specimen of an alien or genetically altered species shall apply to the Council for a permit to do so.”

The purpose of this screening evaluation is to assist the Department of Environment and the National Conservation Council in determining whether the proposed activities pose any potential risk to the natural environment, or pose other risks identified in the Guidance Note, and if so what level of risk assessment will be necessary to inform the Council’s decision on this application.

This taxon concerned is considered “alien” under Guidance Note criteria, which state: *Genetically altered organisms, selectively bred organisms and artificial hybrids are all by definition considered to be alien.*

Background:

This application for operational deployment follows a series of experimental trial releases of this same mosquito strain in Grand Cayman. The first two of these, in East End in 2009 and 2010, pre-dated the passage of the National Conservation Law and so were not subject to the current permitting process. Oxitec conducted its own risk assessment₁ in support of its request to the Cayman Islands Government to conduct these trials.

In June 2016 the Council issued a permit to MRCU & Oxitec to release OX513A males in an area of West Bay, Grand Cayman for a third trial which compared *Aedes aegypti* population trends in the OX513A treated area with a matched control area also in West Bay. This trial was ongoing at the date the Council received the current application.

The current application is for periodic imports of eggs followed by release of OX513A males limited only by a maximum of 2Kg of eggs imported per month. This application is open-ended both in time and geographically within the Cayman Islands, and so should be considered as a permit request for operational deployment of this technology into the indefinite future.

Risk assessments carried out independently of Oxitec have been conducted₂ in response to trials of the OX513A *Aedes aegypti* strain proposed in Brazil, Malaysia and the USA. While these cannot entirely substitute for a risk assessment for the Cayman Islands specifically, there are many commonalities in the receiving environments and the genetically altered mosquito strain is identical in all cases. These pre-existing risk assessments are therefore an important resource directly relevant to this screening assessment.

Oxitec has also submitted a new risk assessment₃ for the current application, meeting the requirements of the NCL Guidance Note. Since this is an output from the applicant, it cannot be considered to be strictly independent, and so its conclusions and any content that is not narrowly factual should be considered carefully in that light.

Information required:

The Guidance Note requires the following information to be submitted by the applicants:

- a) The full scientific name(s) of the specimen(s) proposed to be released.
- b) The numbers and sex ratio of the specimen(s) proposed to be released.
- c) The location(s) and date(s) of proposed releases.
- d) The purpose of the proposed release.
- e) The existing range of this taxon in the wild (including both its native range and its introduced range if any).
- f) If the applicant is a business, a copy of the applicant's current trade and business license is required.
- g) the relevant technical and scientific details as laid out in Paragraph 9 of Annex III of the Cartagena Protocol on Biosafety, under the Convention on Biological Diversity.
- h) a comprehensive statement of the benefits intended to result from the release.
- i) a complete description of facilities and procedures for acquiring and handling the specimens before release, including biosecurity standards and procedures, including procedures for disposal of any substances that may affect the expression of altered gene or genes.
- j) copies of any prior risk assessments that have already been carried out on the genetically altered taxon proposed for release.
- k) copies of any scientific papers relating to risks and benefits associated with the genetically altered taxon proposed for release.

As of 12th April 2017, the applicant has supplied all the required information.

Risk pathways and screening evaluation:

For the purposes of this screening evaluation, the following list of pathways by which the taxon may possibly become invasive and/or harmful has been compiled from prior risk assessments, from

considerations of public claims made by opponents of this technology, from information emanating from the current trial and previous trials, from literature provided by the applicants, and by Cayman Islands specific scenario explorations in discussion among scientific staff at the Department.

The pathways listed here are specific to this application and so do not consider alternative technologies. Pathways involving human health concerns have not yet been locally assessed at the professional epidemiological or medical level, though such expertise is reflected in prior risk assessments from other countries.

1. Local accumulation of Tetracycline (especially from wastewater disposal at the Oxitec facilities) allows larvae arising from escaped or released OX513A males mating with wild (and/or escaped OX513A) females to survive to adulthood, and so start a localized reproducing population of this GM strain. This population would then be subject to very strong selection for any mutation that confers reduced dominance or resistance to the lethal effect.
 - a. Work has been done by the applicant to assess concentration of tetracyclines needed to suppress the activity of the lethal gene, and this is too high to be at all probable in the environment generally. The only possible site(s) of concern in the Cayman Islands would be direct accumulation of discharged water used to rear OX513A larvae. Any extant or future risk could therefore be managed.
 - b. *Aedes aegypti* is characteristically a breeder in clean rainwater accumulated in peri-domestic containers, and in the Cayman Islands is not typically found breeding in septic tanks or wastewater treatment facilities.
 - c. Wastewater disposal at the MRCU Oxitec facility is currently routed to a septic tank shared with the entire MRCU facility. The septic tank water has been analyzed by the Water Authority and is evidently unsuitable for *Aedes aegypti* breeding. A larger, new facility proposed by Oxitec will have its rearing water disposal separated from septic tank uses: no statement has been made as to how this tetracycline-treated waste water will be treated or disposed of.

LIKELIHOOD: Unlikely

CONSEQUENCES: Minor

2. The small percentage of OX513A females that do get accidentally released, carry and transmit diseases capable of affecting wildlife or humans, originating from infected blood used in the OX513A mosquito rearing process.
 - a. Management of this risk would be through conditions that restrict Oxitec to use of synthetic blood. This is a speculative future risk, and probability at present is zero.

LIKELIHOOD: Highly unlikely

CONSEQUENCES: Minor

3. On Cayman Brac, native *Aedes mediovittatus* (which is not particularly closely related to *Aedes aegypti* but is also a competent vector of Dengue virus) hybridizes with released and/or escaped OX513A mosquitoes, and the hybridization process causes the lethal trait to become recessive or inactive. A hybrid population then rapidly evolves and becomes invasive, and could potentially be an efficient new vector of arboviruses.
 - a. *A. aegypti* and *A. albopictus* can hybridize under certain (rare) conditions, but evidence questions whether such hybrids are viable. These two species are both in the subgenus *Stegomyia*. In contrast, *A. mediovittatus* is in the sub-genus *Gymnometopa*. Hybridization should therefore be considered even less likely, but we should not regard it as completely impossible. Tests in captivity could clarify whether or not any risk exists for this eventuality.

LIKELIHOOD: Highly unlikely

CONSEQUENCES: Intermediate

4. Niche vacation by species-specific reduction of *Aedes aegypti* may allow an expansion of invasive *Aedes albopictus*
 - a. Trials to date have not shown this to occur. Monitoring would allow adaptive response if necessary.

LIKELIHOOD: Unlikely

CONSEQUENCES: Minor

5. Proteins arising from the modified genome could be toxic or provoke allergic reactions when mosquitoes bite, or are eaten.
 - a. Other independent risk assessments note this has been tested for extensively, and can now be ruled out as a serious concern.

LIKELIHOOD: Highly unlikely

CONSEQUENCES: Marginal

6. Horizontal gene transfer carries the modified gene(s) into other organisms with unpredictable consequences
 - a. Other independent Risk Assessments point out that natural horizontal gene transfer often occurs between prokaryotes, but natural horizontal gene transfer between eukaryotes, and between eukaryotes and prokaryotes, is practically unknown. In eukaryotes, genetic material is contained within intracellular membranes (nucleus, mitochondria). In prokaryotes the genetic material is free in the cell. It is therefore extremely unlikely that GM components could transfer from the mosquito (which is a eukaryote) to e.g. the bacteria in the mosquito larva's gut (prokaryotes).

LIKELIHOOD: Highly unlikely
CONSEQUENCES: Intermediate

7. Interbreeding between the strain of *Aedes aegypti* used in OX513A and the resident invasive strain, results in the resident population becoming a more efficient disease vector
 - a. For this to occur, the lethal trait in OX513A (which is dominant) would have to be somehow disabled in some proportion of the offspring. The fluorescent marker would show the incursion of the GM components into the wild population, through detection in monitoring larvae. In all trials to date there has been no sign of this happening. Monitoring for this, or evolution of resistance (e.g. as in 1 above) should be a condition of approval.

LIKELIHOOD: Highly unlikely
CONSEQUENCES: Intermediate

8. Bacteria that become resistant to Tetracycline in the rearing facility get introduced into the natural environment, and spread
 - a. The batch processing does not lend itself to long term, successive generations of bacteria evolving in the facility.
 - b. The bacteria that occur in larval rearing water are unlikely to be pathogens of concern to humans or domestic animals, and so the consequence of this may be marginal, and such resistance would not be likely to be adaptively advantageous in the general environment.
 - c. Only if such resistant bacteria then passed their resistance to a pathogenic prokaryote (by horizontal gene transfer) could an actual risk arise – this would seem to be an extremely improbable scenario.

LIKELIHOOD: Highly unlikely
CONSEQUENCES: Minor

Risk Screening Summary:

In the Department's opinion, no identified pathways carry a combination of risk and likelihood sufficient to justify an independent risk assessment under the NCL Guidance Note.

Recommendation:

The Department recommends:

- 1) That the Council be guided primarily by the independent risk assessments referenced below, recognizing these are independent of the applicant and have considerable relevance to the current application.
 - 2) That the Council make conditions should a Permit be granted:
 - a. that a plan for disposal of waste water from all current and future rearing facilities for OX513A in the Cayman Islands is submitted and implemented to the satisfaction of the Water Authority and the Department of Environment, such that no potential exists or can be created for OX513 *Aedes aegypti* to breed in wastewater containing tetracyclines from these facilities.
 - b. that before any OX513A *Aedes aegypti* are transported to or released on Cayman Brac or Little Cayman, tests are conducted by the applicant to establish whether OX513A *Aedes aegypti* are at all capable of hybridizing with *Aedes mediovittatus*, and that expansion of OX313A *Aedes aegypti* releases to the Sister Isles be subject to separate consultation with the NCC after completion of these tests
 - c. that OX513A *Aedes aegypti* eggs transported to the Cayman Islands are produced from females whose blood meals are from synthetic blood only
 - d. that active monitoring for persistence or spread of OX513A *Aedes aegypti* is continued on a permanent, ongoing basis in and around all release areas and around rearing facilities, and that any anomalous occurrences are reported to the Council immediately and investigated by the applicant as a matter of urgency
 - e. that before decision by the Council, the views of the Chief Medical Officer be sought on this application
 - 3) Subject to these conditions and the Chief Medical Officer's views, the Department is of the opinion that given the substantial body of risk assessments that have already been conducted internationally, sufficient information is available to allow the Council to issue a permit if so minded, with precautionary conditions as above, including a requirement for ongoing monitoring.
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Prior Risk Assessments referenced above:

1. Risk analysis – OX513A *Aedes aegypti* mosquito for potential release on the Cayman Islands (Grand Cayman). Oxitec, October 2009.
2. Environmental Assessment for Investigational Use of *Aedes aegypti* OX513A. Centre for Veterinary Medicine, US Food and Drug Administration, April 2016.

Risk Assessment of *Aedes aegypti* strain OX513A. National Technical Biosafety Commission, Brasilia, Brazil. April 2014.

Risk Assessment Report of the Genetic Modification Advisory Committee (GMAC) for an application to conduct a limited mark-release-capture of *Aedes aegypti* (L.) wild type and OX513A strains. Malaysian Department of Biosafety. September, 2010.

3. OX513A Environmental Risk Assessment – Cayman Islands. Oxitec, April 2017.