RISK ASSESSMENT REPORT

OF THE GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC)

FOR

AN APPLICATION TO CONDUCT A LIMITED MARK-RELEASE-RECAPTURE OF

Aedes aegypti (L.) WILD TYPE AND OX513A STRAINS

NBB REF NO: NRE(S)609-2/1/3

APPLICANT: INSTITUTE OF MEDICAL RESEARCH

DATE SUBMITTED: 7 MAY 2010

I - Summary of Assessment Process

The Genetic Modification Advisory Committee (GMAC, please refer to ***Appendix 1*** for details of GMAC), under the purview of the National Biosafety Board was given a Summary of the Application Dossier prepared by the Department of Biosafety on 24 June 2010 for a limited open field trial (release into the environment) involving genetically modified ('GM') mosquitoes of the species *Aedes aegypti,* strain OX513A (My1). The application was filed by the Institute of Medical Research (IMR, hereafter referred to as "the applicant"). It is a joint project with Oxitec Limited, London.

In addition, the Department of Biosafety also provided Entomological Notes (information on the biology of mosquitoes), some selected scientific publications and other relevant reference materials for consideration by GMAC.

After conducting an initial review, GMAC requested for a scientific meeting with the applicant. The two principal researchers of the proposed field trial attended the meeting and gave a scientific briefing on the 6 July 2010. GMAC members also took the opportunity to obtain further clarification on certain details of the proposed field trial. A subsequent meeting was held with the applicant (the 2 principal researchers of the proposed field trial and one researcher from IMR), on the 24 September 2010 where GMAC asked for clarifications on the additional information provided by the applicant.

In order to have a comprehensive assessment and to consider a broad range of Risk Assessment factors, GMAC also requested for meetings with scientists from relevant non-governmental organizations (NGOs) to obtain their views on the proposed field trial. Two NGOs were approached i.e. the Worldwide Fund for Nature (WWF) and the Third World Network (TWN). WWF declined the invitation citing a lack of expertise to comment on the issue. A scientist representing TWN met with GMAC members and discussed several issues including the effectiveness and accuracy of sex selection, gene flow, persistence of GM mosquitoes and larvae in the environment, ecological interactions and the impact on other species, as well as monitoring mechanisms and other related issues such as public consultation and transboundary movement. GMAC took note of these issues and included them, where appropriate, in the risk assessment process.

Further input and information were also sourced from the following:

(i) The Mosquito Research and Control Unit at Cayman Islands, as well as the Department of Agriculture, Cayman Islands. (The collaborator of the applicant, Oxitec. Ltd., had conducted a similar field trial with GM mosquito in the Cayman Islands). GMAC has obtained and reviewed the risk assessment report and summary of results for the Cayman Islands trial.

(ii) The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) Environmental Impact Statement on the "Use of Genetically

Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs". One of the GM pink bollworm strains assessed in the APHIS report contains a transgene construct similar to that used in the GM mosquito in this application (USDA APHIS, 2008).

(iii) The Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) report for "Risk Analysis on the Australian release of *Aedes aegypti* (L.) (Diptera: Culicidae) containing Wolbachia". This document provided useful insights into the risk assessment associated with mosquitoes (Murphy *et al.,* 2010).

The Public Consultation for this application raised some technical and scientific issues regarding the proposed field trial. GMAC considered these comments and addressed those that were not already included in the Risk Assessment.

GMAC had six meetings pertaining to this application and one discussion with a representative from the NGO (Third World Network) from the period of 24 June 2010 until 24 September 2010. Based on the Assessment Process, GMAC has prepared the Risk Assessment Report and Risk Assessment Matrix along with its recommended decision, for consideration by the National Biosafety Board.

II - Background of Application

The project title of the proposed field trial is "Limited Mark-Release-Recapture (MRR) of *Aedes aegypti* (L.) Wild Type and OX513A Strains". It aims to release male GM Yellow Fever mosquito, *A. aegypti* OX513A(My1) strain [hereafter referred to as "OX513A(My1)"] and male non-GM *A. aegypti* mosquito [hereafter referred to as "wild type"]. The purpose of the field trial is (i) to compare and evaluate the longevity and dispersal distance of the male OX513A(My1) in comparison with the wild type, and (ii) to add important information to existing data (based on laboratory and semi-field trials) on the morphology and life history traits of the OX513A(My1) strain.

Previous studies on the OX513A(My1) were conducted as laboratory experiments (contained use) and semi-field trials (conducted in a Temporary Contained Trial Facility at IMR - a fully contained structure, simulating the living space for a household of 2-4 people in Kuala Lumpur). Information had been obtained through bionomic studies to compare OX513A(My1) with wild type, and the mating competitiveness of OX513A(My1) compared to wild type.

It should be noted here that the application is for a **limited release** of OX513A(My1), which is an important prerequisite to any subsequent full scale release involving repeated releases.

Information about OX513A

The parent organism is a transformed *A. aegypti* Rockefeller strain (please refer to Strain Development Diagram in ***Appendix 2***). This was subsequently crossed with a more recently acquired Asian strain of *A. aegypti* at IMR resulting in the OX513A(My1) strain. This strain has been observed to be stable over 60 generations.

The original transformation and subsequent crossbreeding has resulted in OX513A(My1) having two new traits: fluorescence and conditional lethality. The fluorescence trait results in OX513A(My1) having a fluorescent phenotype when excited by illumination of a specific wavelength. This trait is used as a marker as it enables OX513A(My1) and its progenies carrying this transgene cassette to be easily identified in the laboratory and the field. The conditional lethality trait is based on repression of the normal cell cycle of OX513A(My1) in the absence of tetracycline. Hence, when OX513A(My1) mates with either OX513A(My1) or the wild type, the progenies will inherit the conditional lethality trait and die in the absence of tetracycline. In this MRR field trial, only the released OX513A(My1) males will carry the genes for the two new traits. Female mosquitoes in the trial sites that mate with them will produce progenies which will die by the late pupae stage. This, in theory will lead to a decrease in the number of female *A. aegypti* which is normally the vector for the spread of the dengue virus in the next generation.

III - Risk Assessment and Risk Management Plan

GMAC evaluated the application with reference to the following documents:

(i) Roadmap for Risk Assessment of Living Modified Organisms, specifically the additional guidance to conduct a Risk Assessment on Living Modified Mosquitoes (according to Annex III of the Cartagena Protocol on Biosafety produced by the *Ad Hoc* Technical Expert Group (AHTEG) on Risk Assessment and Risk Management of the Convention on Biological Diversity).

(ii) the risk assessment and risk management plan submitted by the applicant.

GMAC in particular, took cognizance of the following as suggested within the AHTEG guidelines:

(i) That the risk assessment exercise should be specific to the details of this particular application

(ii) That the risk assessment exercise should be specific to the receiving environment in question, and

(iii) That any risk identified should be compared against that posed by the unmodified organism.

A Risk Matrix was prepared as a working document based on an assessment mechanism developed by the Health and Safety Department, University of Edinburgh (University of Edinburgh, 2010). For this matrix, GMAC identified potential hazards, and then added a value/rank for the likelihood of each hazard as well as its consequences. The likelihood of each hazard occurring was evaluated qualitatively on a scale of 1 to 4, with 1 for 'highly unlikely', and 4 for 'highly likely'. The consequences of each hazard, if it were to occur, were then evaluated on a scale of 1 to 4, with 1 for 'negligible' and 4 to denote a 'severe consequence'. A value was finally assigned for the overall risk from the identified potential hazard. The general formula: Overall Risk = Likelihood x Consequence was employed. GMAC also proposed risk management strategies for some of the potential hazards. This methodology of assessment follows the procedure of Risk Assessment in Annex III of the Cartagena Protocol on Biosafety.

The Risk Assessment was conducted over a series of six meetings. To start with, the possible pathways to risk/hazard arising from the proposed field trial were identified and listed. The potential hazards identified arose from four main areas:

(i) **Effects on the ecology of the receiving environment**

Issues pertaining to animal health, pollination, food chains, non-target organisms and competition between mosquito species/populations

(ii) **Effects on the biology of the modified mosquito**

Issues pertaining to changes in host range, environment tolerance, lifecycle, reproductive behavior and feeding behavior

(iii) **Effects on human health**

Issues pertaining to toxicity and the possibility of OX513A(My1) becoming a vector of other diseases, and/or causing an increase in disease virulence

(iv) **Effects of the transgene**

Issues pertaining to the stability of the transgene, horizontal gene transfer, changes in expression, interaction and persistence in the environment, toxicity of the gene product/s, mutation and gene silencing.

Based on the above, a final list of 33 potential hazards was identified. Most of these hazards were rated as having an Overall Risk of 1 or "effectively zero" in the **context of a limited MRR field trial**.

GMAC also took extra caution and further discussed pre-emptive mitigation procedures for hazards where the Overall Risk was estimated to be above the minimal, and also for a few hazards that required further evaluation and data acquisition. Some of these risks are expected to be managed effectively with the risk management strategies proposed (please refer to section IV of this document). Taking into consideration the limited scale of the release (in terms

of total number of mosquitoes released, number of releases, and number of sites involved), some of the identified hazards were found to be highly unlikely in the present MRR field trial. However, these issues should be thoroughly reassessed if there is a subsequent application for a larger scale release.

Two pertinent potential hazards where the Overall Risk was found to be above 1 are highlighted below along with the appropriate management strategies:

a) Possibility of OX513A(My1) females mosquitos being unintentionally released

Sorting of mosquitoes to isolate only males is first done mechanically based on pupae size. Following this, three senior laboratory technicians cross-check serially to eliminate any females that might have been missed. To minimize sorting error resulting in the possible unintended release of OX513A(My1) females, the submitted Standard Operating Procedure (SOP) should be followed strictly. The same SOP will also be used to sort the wild type mosquitoes. GMAC has reviewed the SOP submitted by the applicant for this process.

b) Introduction of the transgene into the wild population due to reduced gene penetrance

A possible 3% survival rate of OX513A(My1) progeny can be expected. There is concern that these surviving OX513A(My1) mosquitoes will be able to breed with the wild population and the transgene transmitted to the wild population. However, the overall risk of this occurring has been identified as Low/2 as it is likely to be overcome by the process of natural selection. In general, highly inbred laboratory organisms, such as OX513A(My1), have low survival fitness. Furthermore, OX513A(My1) has no selective survival advantage and since it is present in very small numbers, will diminish quickly in the wild population. Even so, to minimize the risk of any residual OX513A(My1), GMAC requires the applicant to carry out a comprehensive fogging, capturing, clean-up and monitoring operation. The monitoring period has also been extended for one month beyond the end date of the field trial.

IV - Proposed Terms and Conditions for Certificate of Approval

Based on the 33 potential hazards identified and assessed, GMAC has drawn up the following terms and conditions for the certificate of approval for the MRR field trial:

Part A

Information and/or documentation that should be submitted to NBB at least two weeks prior to the start of field trials

a) Documentation from District Council/*Majlis Daerah* or relevant authorities on the presence or otherwise of aquaculture, poultry and pharmaceutical industries within a vicinity of 500 meters of the release sites, and information on whether any of these industries regularly use tetracycline[1] in their operations [This is related to the concern that there may be residual tetracycline around the release sites.]

b) Confirmation from the relevant health authorities that the sites selected has been free from any dengue outbreak for at least 3 months before the start of the field trial.

c) Detailed information on the positioning of the ovitraps and BG-Sentinel traps (documentation on setting-up of traps, including GPS information and photographs has been proposed by the applicant). Proper cautionary measures should be taken to ensure that that traps are positioned at suitable locations/heights for effective trappings.

d) A consent letter should be provided from the Local Council for the district/s where the release sites are located for the proposed MRR field trial.

e) Public Notification and Consensus - It is mandatory that the applicant through a public forum obtains prior consensus and approval from the inhabitants in the release sites regarding the proposed MRR field trial.

[1] Residues of tetracycline in water discharged from agricultural activities, especially aquaculture and poultry farming, or *via* pharmaceutical industries, etc. could enable OX513A(My1) progeny to survive if the level is above the threshold. However the risk is low as *A. aegypti* does not lay eggs in discharged, polluted or running water. Furthermore, tetracycline has a short lifespan and is light and heat sensitive. However, for this field trial, the applicant is required to provide proof that there are no aquaculture, poultry and pharmaceutical industries that use tetracycline or its derivatives in the vicinity of the release site/s.

Part B

Actions to be taken and reported to NBB during /after the field trial

a) All proposed activities and methods submitted in the dossier and agreed upon through other means of communication with the applicant should be appropriately and responsibly adhered to.

b) Sex sorting must be carried out in compliance with the SOP submitted (SOP for Sex Sorting of *Aedes aegypti* Mosquitoes). Additionally, all OX513A(My1) mosquitoes for release must be checked and not merely a 'quality control sample'.

c) All extra insects/ recaptured insects are to be transported in shatter-proof double-covered containers for subsequent identification, analytical studies or appropriate disposal (according to SOP at IMR.)

d) At the end of the field trial, fogging[2] for a 400m radius is required according to the Ministry of Health's guidelines. In addition, clean-up operations (*gotong-royong*) should be conducted at the end of the field trial to eradicate all breeding grounds. A second fogging should be conducted one week after the end-of-field-trial fogging.

e) At the end of the field trial (first fogging), the applicant is required to continue monitoring for another month to ensure no residual OX513A strains are left behind. The traps should be checked on a daily basis. During this additional one month monitoring period, fogging should be done if any residual OX513A(My1) is detected.

f) Upon completion of the open field trial, a comprehensive report should be submitted to the National Biosafety Board within two months from the end of the trial.

V - Other Regulatory Considerations

GMAC recommends that there should be a monitoring mechanism throughout the field trial period to ensure full compliance with the agreed terms and conditions by the applicant.

[2] In order to ensure that no residual OX513A(My1) mosquitoes remain, the applicant has proposed for fogging to be carried out after the one month period. The proposed control mechanism has been tested and proven to be effective with *A. aegypti*, and also for eradication of OX513A(My1) strain. Fogging will also be used as the Emergency Response Plan.

VI - Identification of Issues to be Addressed for Future Releases

Some additional issues have been identified that would be important during the assessment of an application for a larger scale or commercial release of OX513A(My1) mosquitoes. These include:

a) Risk: Release of OX513A(My1) mosquitoes may cause other pests to become more serious

The current proposed release involves a relatively small number of OX513A(My1) mosquitoes and the duration of the field trial is short. However, for a larger release, this risk should be considered more thoroughly.

b) Risk: Increase in the population of another mosquito species due to suppression of the target mosquito

Proper baseline data on, and close monitoring of, any change in the populations of *A. aegypti* and other mosquito species are required as a release of a larger number of mosquitoes is likely to affect these populations.

c) Integrated Pest Management (IPM) programme

For a large scale release to control the population of *A. aegypti,* an IPM programme should be in place to further augment the technology.

d) Stability of the transgenes in the field

Molecular information has to be obtained on the stability of the transgenes through multiple generations in the field. This includes the independent stability of expression of the tTAV gene with or without expression of the DsRed marker gene and the stability of the transgene cassette.

e) **Behaviour of OX513A(My1) in the field**

The behaviour (e.g. mating, biting, etc.) of OX513A(My1) in the field has to be assessed in comparison to its behavior in containment.

f) **Sorting error**

The effectiveness in screening and elimination of female mosquitoes should be ensured even when larger numbers are involved, especially since human error could then adversely affect the process.

VII – Conclusion and Recommendation

GMAC has conducted a thorough evaluation of the application entitled Limited Mark-Release-Recapture (MRR) of *Aedes aegypti* (L.) Wild Type and OX513A Strains and has determined that the field trial does not endanger biological diversity or human, animal and plant health.

GMAC recommends that the proposed field trial be **APPROVED WITH TERMS AND CONDITIONS** as listed in section IV - Proposed Terms and Conditions for Certificate of Approval, Parts A and B.

GMAC also recommends that section VI - Identification of Issues to be Addressed for Future Releases be forwarded to the applicant for further reference.

VIII - Bibliography

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APPENDIX 1

GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC) MEMBERS INVOLVED IN SPECIFIC RISK ASSESSMENT AREAS FOR FIELD EXPERIMENT OF TRANSGENIC MOSQUITOES

Genetic Modification Advisory Committee (GMAC) members have divided the task of looking up more information for the Risk Assessment matrix based on four broad categories. The scope of research aspects for each group is as listed below. Each sub-committee has a nominated leader to coordinate the work and report back to the main GMAC. The respective leader will contact the sub-committee members and discuss the work process with their members. The groupings of GMAC sub-committee members and the assigned tasks are as below:

1) Effect on ecology of receiving environment (e.g animal health, pollination, food chain, non target organism, competition between mosq. sp/pop studies)
    - **Dr. Ahmad Parveez bin Hj Ghulam Kadir (Malaysian Palm Oil Board)(Leader)**
    - Dr. Wan Abdul Rahaman bin Wan Yaacob (BiotechCorp)
    - Madam Atikah binti Abdul Kadir Jailani (Department of Agriculture)

2) Effect on biology of mosquito (e.g change in host range, change in environment tolerance, lifecycle, reproductive behaviour, feeding behaviour)
    - **Dr. Tan Swee Lian (Malaysian Agricultural Research & Development Institute)(Leader)**
    - Assoc. Prof. Dr Jothi Malar Panandam (Universiti Putra Malaysia)
    - Prof. Dr Son Radu (Universiti Putra Malaysia)

3) Effect on human health (e.g toxicity, vector of other diseases, increase virulence)
    - **Dr. S. Ravigadevi (Malaysian Palm Oil Board)(Leader)**
    - Madam Shamsinar binti Abdul Talib (Ministry of Health)

4) Effect of transgene (stability, horizontal gene transfer, change in expression, interaction, persistence in environment, toxicity of gene product, mutation, silencing,)
    - **Assoc. Prof. Dr. Mohd. Faiz Foong bin Abdullah (Universiti Teknologi MARA) (Leader)**
    - Dr. Tan Chon Seng (Malaysian Agricultural Research & Development Institute)
    - Prof. Dr. Helen Nair (Academy of Science Malaysia)
    - Dr. Chow Keng See (Malaysian Rubber Board)

APPENDIX 2

STRAIN DEVELOPMENT DIAGRAM