FACT SHEET

NATIONAL BIOSAFETY BOARD DECISION ON THE APPLICATION FOR APPROVAL FOR LIMITED MARK-RELEASE-RECAPTURE OF *Aedes aegypti* WILD TYPE AND *Aedes aegypti* GENETICALLY MODIFIED MOSQUITOES OX513A(My1)

NBB REF NO: NRE(S)609-2/1/3
APPLICANT: INSTITUTE OF MEDICAL RESEARCH
DATE OF DECISION: 5 OCTOBER 2010

The objective of the Biosafety Act 2007 is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) has assessed an application for approval submitted by the Institute of Medical Research (IMR) to release genetically modified male Yellow fever mosquitoes (*Aedes aegypti*) for the purpose of a field trial. The NBB on the 5th October 2010 made a decision to grant an **approval with terms and conditions** to conduct this field trial.

1) **What is this approval for?**

   The approval with terms and conditions is for IMR to conduct a field trial (release into the environment). The project title is "Limited Mark-Release-Recapture (MRR) of *Aedes aegypti* (L.) Wild Type and OX513A strains". Approval is given to release genetically modified male Yellow fever mosquitoes (*Aedes aegypti*), which is referred to as *Aedes aegypti* OX513(My1) strains.

2) **What is the purpose of the field trial?**

   The purpose of the field trial is to compare and evaluate the longevity and dispersal distance of the male *Aedes aegypti* OX513(My1) strain in comparison with the wild type strain (non genetically modified mosquitoes). This field trial will help in adding important information to existing data on the morphology and life history traits of the OX513(My1) strain. Previous studies have been conducted on the OX513(My1) strain in laboratory experiments (contained use) and semi field experiments (experiments were conducted in a Temporary Contained Trial Facility - a fully contained structure, simulating the living space for a household of 2-4 people in Kuala Lumpur). If this technology is proven to be successful through field trials, it may be used as part of an Integrated Pest Management Programme to curb dengue.

3) **How has the *Aedes aegypti* OX513(My1) strain been modified?**

   The *Aedes aegypti* OX513(My1) strain has been modified to include 2 new traits, fluorescence and conditional lethality. The fluorescence trait allows the OX513(My1) strain mosquitoes to have a fluorescent phenotype when excited by illuminations of a specific wavelength. This trait is used as a marker as it enables the OX513(My1) to be easily identified in the laboratory and field. The conditional lethality trait represses the normal cell cycle of

the mosquito in the absence of tetracycline. Hence, when OX513(My1) strains mate, progenies that arise from this mating will inherit the gene and express the trait, resulting in the death of the progenies in the absence of tetracycline.

4) Information about release site of the *Aedes aegypti* OX513 strains?

The release will take place in Pahang (Bentong district) and Melaka (Alor Gajah and Melaka district). Each location will have 2 release phases. The first phase will be a release at an uninhabited site approximately 0.5-1 km from the nearest human population and the second phase will be a release at an inhabited site. The size of the proposed sites can be up to 5km^2. Releases will be carried out from a single point. A limited release of approximately 2,000-3,000 OX513(My1) strains a day for 2 consecutive days or a single release of approximately 4,000-6,000 OX513(My1) strains alongside the release of an appropriate number of wild type *Aedes aegypti* will be conducted in both inhabited and uninhabited sites. The field trials may be repeated.

5) Characteristics of the *Aedes aegypti* OX513(My1) strains

Disease transmission

Only male *Aedes aegypti* OX513(My1) strains are released. Male mosquitoes **do not have the morphology to bite and do not carry any disease.** The anatomical structure of the mouthparts and the anatomical structure of the stomach prevent the male mosquitoes from biting. Therefore, male mosquitoes do not transmit disease or take a blood meal. Additionally anti-coagulants secretions are required for biting and this has not been observed in males.

Gene transfer

Exchange of genetic materials between different insect species in the natural environment rarely happens as insects exchange gametes internally and have complex mating behaviours and structures to prevent interspecies mating. Hence, mosquitoes are not capable of interbreeding with other insect species. The laboratory condition inter-species mating that were conducted between the closely related species, *Aedes aegypti* and *Aedes albopictus* resulted in no fertile hybrids.

Furthermore, the repressible lethal gene conferred to OX513(My1) *Aedes aegypti* confers a selective disadvantage to the organism, therefore highly unlikely to be maintained in an organism in the unlikely event that genetic material is transfered by horizontal gene transfer.

Fitness of the OX513(My1) *strain*

The characteristics and fitness of the OX513(My1) strain has been observed in the laboratory and under semi-field conditions (using field house that replicates typical Malaysian dwelling) in comparison to the wild type *Aedes aegypti*. There were no significant differences in terms of number of eggs laid, egg hatching rate, number of larvae hatched, number of pupae in F1 (first) generations, larval survivorship, pupation, adult eclosion rate, number of days in each stage of life cycle, gonotrophic cycle and adult fecundity between the OX513(My1) strain and the wild type *Aedes aegypti*.

Susceptibility of OX513(My1) strains to standard adulticides

The susceptibility of OX513(My1) strains to standard adulticides including those used as main active ingredients for mosquito control in Malaysia (e.g. malathion, lambda-cyhalothrin, propoxur, DDT) have been tested. The OX513(My1) mosquitoes were susceptible to all the chemicals tested, except DDT (this chemical is not in use anymore). The current standard adulticides will be effective at control of OX513(My1) strains when used at standard concentration.

Effects on non target organisms

Studies were conducted to determine the effects on an organism feeding exclusively on OX513(My1). The organism chosen was *Toxorhynchites splendens* (a mosquito in the same family as *Aedes aegypti*) as *Toxorhynchites* larvae are predatory on other invertebrate species such as *Aedes* or other mosquitoes.

OX513(My1) larvae that had been reared both on and off tetracycline were fed to *Toxorhynchites* larvae as 100% of their diet. The experiment was conducted over 6 successive generations of *Toxorhynchites* and results showed no evidence of any difference between *Toxorhynchites* fed on OX513(My1) and the wild type control. Also, no toxic elements have been incorporated into the OX513(My1), so potential hazard arising from the dead insects persisting in the environment is highly unlikely.

6) What controls are proposed for this release?

Through the risk assessment and risk management process it has been concluded that, the release poses no risks to human and the environment. Only male mosquitoes are released and male mosquitoes do not bite or carry the dengue virus. The Standard Operating Procedures for sorting the male mosquitoes for the release has been assessed and approved by the Genetic Modification Advisory Committee (GMAC) of the NBB. Sorting of mosquitoes to isolate only males is first done mechanically based on pupae size. Sorting will be done mechanically, followed by a serial manual re-check on all the sorted mosquito pupaes by three highly trained laboratory technicians of IMR. The same SOP will also be used to sort the wild type mosquitoes.

Control measures in order to prevent the *Aedes aegypti* OX513(My1) strains and genetic material from being persistent in the environment have also been proposed. These include placing traps to recapture the mosquitoes and continuous daily monitoring of the traps until no marked mosquitoes are recaptured for 3 successive days. For the inhabited trial sites, the sites will be fogged with appropriate insecticide after the completion of the release and trapping. Upon completion of the field trial, fogging for a 400m radius is required according to the Ministry of Health's guidelines and a clean-up operations (*gotong-royong*) should be conducted to eradicate all breeding grounds. A second fogging should be conducted one week after the end-of-field-trial fogging. IMR will continue to monitor the site for an additional one month to ensure no residual OX513A(My1) strains are left behind. The traps should be checked on a daily basis. During this additional one month monitoring period, fogging will be done if any residual OX513A(My1) is detected.

All unused insects, recaptured insects, will be transported in shatter-proof double containers for identification, further analysis and appropriate disposal according to SOP at IMR.

In addition, due to the conditional lethality trait of the OX513(My1) strain, the progenies of this strain will die, hence limiting or eliminating the possibility of the gene being persistent in the environment or transferring to other organisms.

7) What is the Emergency Response Plan?

Fogging or treating the area with insecticides. The type of chemical used is Resigen® and follows the standard procedure of the Ministry of Health in conducting fogging.

8) What's Next?

IMR must obtain prior consensus from the inhabitants/public in the trial site/s through a public forum before proceeding with the field trial. In addition, IMR also has to fulfil the conditions imposed with the approval, whereby some information and/or documentation should be submitted to NBB at least two weeks prior to the start of field trials.

NBB, through the Department of Biosafety, will closely monitor the implementation of the field trial to ensure compliance at every stage of the release.