FACT SHEET

APPLICATION FOR APPROVAL FOR LIMITED MARK-RELEASE-RECAPTURE OF *Aedes aegypti* (L.) WILD TYPE AND OX513A STRAINS

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

The objectives of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, The National Biosafety Board (NBB) is currently assessing 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 field experiment.

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

   To conduct a field experiment (release into the environment). The project title is "Limited Mark-Release-Recapture (MRR) of *Aedes aegypti* (L.) Wild Type and OX513A strains". The application is to release genetically modified male Yellow fever mosquitoes (*Aedes aegypti,)* which is referred to as *Aedes aegypti* OX513 strains.

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

   The purpose of the trial is to compare and evaluate the longevity and dispersal distance of the male *Aedes aegypti* OX513A 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 OX513A strain. Previous studies have been conducted on the OX513A 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 experiments, it may be used as part of an Integrated Pest Management Programme to curb dengue.

3) **How has the *Aedes aegypti* OX513A strain been modified?**

   The *Aedes aegypti* OX513A strain has been modified to include 2 new traits, fluorescence.and conditional lethality. The fluorescence trait allows the OX513A 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 OX513A 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 OX513A 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**?

   If approved, the release which is expected to commence on second week of October or November 2010 and 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 OX513A strains a day for 2 consecutive days or a single release of approximately 4,000-6,000 OX513A strains alongside the release of an appropriate number of wild type *Aedes aegypti* will be conducted in both inhabited and uninhabited sites. The experiments may be repeated.

5) Characteristics of the *Aedes aegypti* OX513 strains

Disease transmission

Only male *Aedes aegypti* OX513 strains are released. Male mosquitoes do not have the morphology to bite. The anatomical structure of the mouthparts and the anatomical structure of the stomach prevents 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

According to the applicant, 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 was conducted between the closely related species, *Aedes aegypti* and *Aedes albopictus* resulted in no fertile hybrids.

Furthermore, the repressible lethal gene conferred to OX513A *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 OX513A strain

The characteristics and fitness of the OX513A 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 OX513A strain and the wild type *Aedes aegypti*. Only one parameter differed which was the post-emergence adult longevity. The post-emergence adult longevity for the wild type strain exceeded the OX513A strain whereby the mean lifespan for the wild type strain was 25.67 days and 20.00 days for the OX513A strain. This may be due to a fitness penalty associated with the transformation of the strain.

Susceptibility of OX513A strains to standard adulticides

The susceptibility of OX513A 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 OX513A 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 OX513A strains when used at standard concentration.

Effects on non target organisms

Studies were conducted to determine the effects on an organism feeding exclusively on OX513A. 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.

OX513A 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 OX513A and the wild type control. Also, no toxic elements have been incorporated into the OX513A, so potential hazard arising from the dead insects persisting in the environment is highly unlikely.

6) What controls are proposed for this release?

Through their risk assessment and risk management, the applicant declared that the release poses no risks to human and the environment. Control measures in order to prevent the *Aedes aegypti* OX513 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. If marked mosquitoes are still being caught after 1 month, further trapping can be put in place. For the inhabited trial sites, the sites will be fogged with appropriate insecticide after the completion of the release and trapping. Fogging will be carried out in houses within 200m radius of the release point. 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. It is also proposed to fog the area with appropriate insecticides one month after the release.

In addition, due to the conditional lethality trait of the OX513A 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 other insecticides is proposed by the applicant. The type of chemical used is Resigen® and follows the standard procedure of the Ministry of Health in conducting fogging.

8) How can I comment on this application?

Any member of the public may submit their comments or queries on a publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Your comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed

by the proposed release are appreciated. The submission of the comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on 4 September 2010 and written submissions are required by that date. Submissions must be addressed to: The Director General, Department of Biosafety, Ministry of Natural Resources and Environment, Level 1, Podium 2, Wisma Sumber Asli, No. 25, Persiaran Perdana, Precinct 4, 62574 Putrajaya, MALAYSIA. Email: biosafety@nre.gov.my. Fax No.: 03-88904935.

Please include your full name, address and contact details in your submission.