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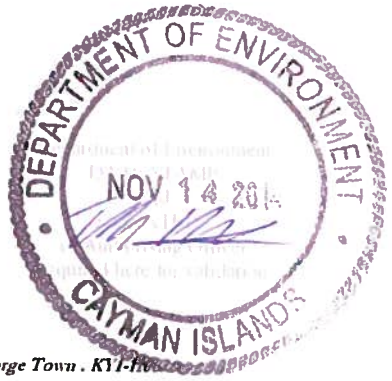

DEPARTMENT OF ENVIRONMENT
CAYMAN ISLANDS GOVERNMENT

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CAYMAN ISLANDS
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APPLICATION to CONDUCT SCIENTIFIC STUDY in the CAYMAN ISLANDS

Once date-stamped by the Department of Environment, this proposal constitutes an agreement between the following Parties:

Department of Environment

(on behalf of Cayman Islands Government, and the People of the Cayman Islands)

and

The Applicant

The Applicant (and in the case of student applicants, their Study Supervisor), undertakes to conduct scientific research and share information, as follows:

APPLICANT NAME	**Shmona Simpson**
(SUPERVISOR NAME)	**Dr. William Petrie**
PROJECT TITLE	**Implementation of an Operational Program based on Oxitec RIDL Technology for the Control and Potential Elimination of the *Aedes aegypti* Mosquito Vector of Dengue and Chikungunya Fever in an Area of Grand Cayman**
INSTITUTION	**Mosquito Research & Control Unit**
POSITION	**Senior Research Officer**
DATES IN CAYMAN	**July 2014 – July 2015**

CONTACT NAME	**Shmona Simpson**
ADDRESS	**Marco Giglioli Centre** **99 Red Gate Road**

	P.O. Box 486 **Grand Cayman KY1-1106** **Cayman Islands**
PHONE	**Tel: (345) 949-2557 Fax: (345) 949-8912**
EMAIL	**Shmona.simpson@gov.ky**

By signing my initials to each line,

I, **The Applicant,** confirm that I have read, understand, and agree to the following:

		Applicant initials
1	I will conduct my research as described in this application, and will abide by the terms set forth in this agreement.	SS
2.	I will obtain community approval through contact with the Cayman Islands Department of Environment, who will assist in the further approach to the community if they feel the work is appropriate.	SS
3.	I agree that research should be an educational process leading to mutual learning among researchers, collaborating individuals, communities and institutions. I agree to share the results of my research in a timely manner with such, both directly and through the Department of Environment.	SS
4.	I agree to include appropriate acknowledgement of collaborating researchers, individuals, communities and institutions in any publications arising from my research, through agreed acknowledgements, citations, authorship, and inventories as applicable, and likewise respecting requests for anonymity.	SS
5.	I agree that, just as the propriety rights of scientific knowledge are well established and respected; such rights are due to the producers and providers of traditional knowledge and contemporary innovations from local communities.	SS
6.	I agree that my activities will respect local cultural values and norms.	SS
7.	I agree that any benefits accrued from my research will be shared with all partners in a fair and equitable manner.	SS
8.	I agree to follow all procedures required by the Cayman Islands Government Department of Environment, to abide by local and international laws, and by the Guiding Principles of the Environment Charter (appended).	SS
9.	I agree to explain the nature and purpose of the proposed research, including its duration, the geographic area in which it will take place, and collecting methods.	SS
10.	I agree to explain the foreseeable consequences of the research, for resources, people and stakeholders, including potential commercial value and non-commercial values, such as academic recognition.	SS
11.	I agree to explain any guidelines that my research is following, as well as my previous involvement in similar research projects.	SS
12.	I agree to provide, upon request, copies of relevant project documentation, or summaries thereof, in English. In the case of commercial prospecting, researchers must provide such documents, including budgets.	SS
13.	I agree to share findings at different stages with the providers, on request.	SS
14.	I agree to not engage in bribery or the making of false promises.	SS
15.	I agree to provide, on request, a preliminary description of findings before leaving the community.	SS
16.	I agree to provide a written report on the research to the Cayman Islands Department of Environment, as soon as possible, with an executive summary (at least) in English, and that if the research is on-going, to provide periodic reports (at least annually) to the Cayman Islands Department of Environment.	SS
17.	I agree to send a copy of any publications arising out of this research to the Cayman Islands Department of Environment.	SS
18.	I agree, upon request and at reasonable time, to provide access to original collected data to the Cayman Islands Department of Environment.	SS
19.	I understand that once issued, a permit will be subject to periodic review, and can be revoked at any time if this agreement is violated.	SS

The Department of Environment undertakes to assist approved projects with paperwork and permitting, advice, access to resources, communications and logistics, where possible. DoE maintains subsidized accommodation on Grand Cayman and Little Cayman which may be available for use by Visiting Scientists.

1. STUDY SPECIES

Transgenic *Aedes aegypti* and *Aedes albopictus*

2. PROJECT PURPOSE

The mosquito-borne diseases Dengue Fever and Chikungunya Fever are transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. These diseases generally result in symptoms such as fever, headache, vomiting, diarrhea, rash and joint pain. Rarely, in severe and complicated cases, chronic arthritis, haemorrhagic (bleeding) fever, and the fatal dengue shock syndrome, can occur.

It is not possible to prevent the introduction of such diseases to the Cayman Islands; hence MRCU currently conducts adulticiding and larviciding of all mosquito species across the Cayman Islands to prevent transmission. However, insecticide resistance is increasing. It is conventionally accepted that novel strategies will have to be employed alongside traditional methods to control mosquito-borne illness.

Scientists at Oxitec (www.oxitec.com), a UK-based biotech firm have inserted a lethal gene into male *Aedes aegypti* and *Aedes albopictus* mosquitoes. The plan is to release males only because they do not bite. When released into the environment, these genetically modified males compete with wild males for wild females. If the GM males mate with the wild females, they pass on a deadly gene that leads to the death of their offspring.

Once enough of these modified males are released over a period of time, the numbers of *Aedes aegypti* can be reduced significantly. In fact, recent studies in Brazil show suppression of *Aedes aegypti* by up to 96%. Suppression of Aedes aegypti and Aedes albopictus to this extent could greatly reduce the risk of transmission of Dengue and Chikungunya Fever transmission in the Cayman Islands.

The immediate or direct benefit that the Project will achieve - the rationale for doing the Project.

3. PROJECT OUTPUTS

600,000 transgenic males would be released every week for a nine month period in West Bay. It is expected that the total number released would be around 22 million males, with the aim of achieving 80% reduction in the numbers of *Aedes aegypti* within the treatment area.

The deliverables or products of the Project Activities - these should be sufficient to deliver the Project Purpose.

4. PROJECT ACTIVITIES

Rearing and Sorting

Mosquitoes will be reared in an ACL-2 compatible Mobile Research Unit based on the MRCU site at Red Gate Road. Eggs will be vacuum hatched. Larvae reared in trays containing 1 L of water and fed daily with Vipan fish food. After pupation, larvae and pupae will separated, first mechanically on the basis of size using specialised equipment. Pupae will then be separated into males and females again on the basis of size using a wire sorter, and the males be allowed to eclose into a cage. The emerged male adults will be provided with 10% sugar solution supplemented with vitamin B complex solution until their release.

Rearing of Males

OX513A males will be released at an average of 465 males/ha/week. There are several compelling reasons to release only males, one being that only female mosquitoes bite humans. Release will be conducted four days after pupal sorting when the males are sexually mature.

Recaptures and Monitoring

Ovitraps (which mimic natural oviposition sites in which females lay eggs) and BG-Sentinel adult

traps (Biogents) will be used in and around the release area; and an equivalent untreated control site. This specialised monitoring will be conducted alongside routine island-wide MRCU monitoring and control activities for all mosquito species. Ovitraps will be serviced weekly, BG Sentinels weekly or daily at different stages of the experiment. Eggs from ovitraps will be hatched under vacuum and the resulting larvae analysed.

Main activities planned within the project - these should be sufficient to deliver the Project Outputs.

5. STUDY LEAD, PROJECT PARTNERS and FUNDING SOURCE

The study is conducted jointly by the Mosquito Research and Control Unit under the auspices of the Ministry of Health, Cayman Islands Government; and, Oxitec, a British Biotech company with a mandate to develop and conduct research around novel technologies to control the spread of vector-borne illness. The study is funded entirely by the Cayman Islands Government.

6. PROJECT DELIVERABLES

A report will be produced at the end of the study detailing the project outcomes and future plans. It is anticipated that the results of this study will be published in high-impact scientific journals.

To be provided to DoE / local partners where appropriate – (e.g. reports, papers, specimens, acknowledgements)

7. INTERACTIONS with STUDY SPECIES

Studies have shown that the transgenic males die within a few days. Monitoring from previous research also shows that after two weeks, there is no evidence of the transgene (the gene introduced) in the field.

8. CONSERVATION: POSITIVE CONTRIBUTIONS / NEGATIVE IMPACTS

Aedes aegypti and *Aedes albopictus* are not native to the Cayman Islands. They were introduced. Thus, there should be no birds, fish or other insects that depend on it for their survival nor does it perform services like pollinating flowers. *Aedes aegypti* is closely associated with man and an urban environment, breeding mainly in man-made containers and almost exclusively feeding on man. Therefore, reducing the number of *Aedes* is most unlikely to have a negative impact on the environment.

This is a self-limiting strategy, whereby the released mosquitoes will die in the environment and the transgene (the gene introduced) will rapidly disappear from the environment following cessation of releases, as no progeny that inherit it survive to adulthood.

Animals that eat the sterile *Aedes aegypti* mosquito will be exposed to nutritional elements – protein, fat, sugar and others - as they would from eating any mosquito: they cannot take up genes through this route, and the gene which been introduced affects only the offspring of the modified mosquitoes. It works by causing subtle adjustments to their cells in a way that is not toxic to organisms that eat those cells, including the bacteria and fungi that decompose dead insects.

Though the primary target of this technology is *Aedes aegypti* because there are significant numbers in the Cayman Islands, we plan to carefully monitor the numbers of *Aedes aegypti* and *Aedes albopictus* during this trial. Should *Aedes albopictus* begin to occupy the *Aedes aegypti* niche upon reduction in their numbers, a concurrent operation will begin to reduce the numbers of *Aedes albopictus*.

9. HEALTH and SAFETY CONSIDERATIONS / MEASURES TAKEN

Tetracycline in the Environment:

Tetracycline is the antidote to the lethal technology, whereby if transgenic larvae are reared in the presence of tetracycline- they will survive to adulthood.

An estimated a threshold level of between 0.01 and 0.1ug/ml of tetracycline is required to see an effect on survival of the mosquitoes in the laboratory.

The literature suggests that there is an average concentration of 0.00066ug/m in the environment.

This value is substantially less than the amount required for survival of the RIDL mosquitoes.

Further reading:

Duff, B. (2005). Presence of tetracycline antibiotics in surface water. A study of the presence/ absence of tetracycline in the Raccoon river watershed, Des Moines water works laboratory.

Gulkowska, A., Y. He, et al. (2007). "The occurrence of selected antibiotics in Hong Kong coastal waters." Mar Pollut Bull 54(8): 1287-1293.

Le-Minh, N., S. J. Khan, et al. (2010). "Fate of anitbiotics during municipal water recycling treatment processes. ." Water Research 44: 4295-4323.

Locatelli, M. A. F., F. F. Sodre, et al. (2011). "Detemination of Antibiotics in Brazilian Surface Waters Using Liquid Chromatography-Electrospray Tandem Mass Spectrometry." Arch Environ Contam Toxicol 60: 385-393.

McQuillan, D., S. Hopkins, et al. (2002). Drug Residues in Ambient Water: Initial Surveillance in New Mexico, USA. 7th Annual New Mexico Environmental Health Conference.

Affect on Population Health

Exposure will be minimised to biting transgenic mosquitoes. All female pupae that are sorted will be frozen and disposed of. Though the aim is to only release males which do not bite, there is a small chance that some females will be released. Previous results from Cayman showed that less than 1 in every 1500 males released was a female. The Mobile Research Unit is built to ACL2 standards of containment to prevent unintentional release around the unit. There is a double door entry with secure access, a sieve to prevent any biological material from escaping and all waste is frozen to kill any insects and then incinerated.

Should a person be bitten by a transgenic mosquito, the risk to human health or the environment from this release is likely to be minimal. If a person were bitten by a female sterile mosquito it would be exactly the same as a bite from a wild one, in fact rather less dangerous in several respects - released mosquitoes will be free from disease, and very few transgenic females are likely to survive long enough to transmit disease once in the environment. There is no evidence at this time to indicate that any of the proteins expressed in the transgenic mosquitoes are likely to be allergenic to humans.

10. IMPORT / EXPORT REQUIREMENTS

We will import about 20 million *Aedes aegypti* transgenic eggs, and an appropriate amount of *Aedes albopictus* eggs if required. There will be no planned export to other jurisdictions, and we do not anticipate a risk of accidental export to other jurisdictions.

Environment Charter

CAYMAN ISLANDS

CAYMAN ISLANDS

Guiding Principles

UK Government, for the government of the Cayman Islands and for the people of the Cayman Islands

1 To recognise that all people need a healthy environment for their well-being and livelihoods and that all can help to conserve and sustain it.

2 To use our natural resources wisely, being fair to present and future generations.

3 To identify environmental opportunities, costs and risks in all policies and strategies.

4 To seek expert advice and consult openly with interested parties on decisions affecting the environment.

5 To aim for solutions which benefit both the environment and development.

6 To contribute towards the protection and improvement of the global environment.

7 To safeguard and restore native species, habitats and landscape features, and control or eradicate invasive species.

8 To encourage activities and technologies that benefit the environment.

9 To control pollution, with the polluter paying for the prevention or remedies.

10 To study and celebrate our environmental heritage as a treasure to share with our children.

W. McKeeva Bush
CAYMAN ISLANDS
26 September 2001

Valerie Amos
UNITED KINGDOM
26 September 2001

I, **The Applicant,** undersigned, confirm that I have read, understand, and agree to abide by the agreement outlined in this document.	
SIGNATURE OF APPLICANT	**DATE**

FOR OFFICIAL USE ONLY:	
PERMISSION GRANTED	**YES / NO**
REASON As outlined in attached 'Recomendations to Import & Release',	
NAME OF PERMIT ISSUER John Bothwell Senior Research Officer	
SIGNATURE OF PERMIT ISSUER	
DATE 14 NOV 2014	
DATE STAMP DEPARTMENT OF ENVIRONMENT NOV 14 2014 CAYMAN ISLANDS	

Follow-Up information relating to MRCU's request to release genetically modified *Aedes aegypti* and *Aedes albopictus* mosquitos.

1) DoE Questions after initial application.
    a. MRCU supplemental answers.

1) How (if) MRCU will track GM mosquito dispersal through the study site and beyond?
    a. Ovitraps mimic natural oviposition sites in which females lay eggs and BG-Sentinel adult traps (Biogents) chemically attract all mosquitoes species towards them. The mosquitoes are subsequently trapped.
    b. We will be using both of these recapture methods in and around the release area; and an equivalent untreated control site. This is specialised monitoring which is conducted alongside routine island-wide MRCU monitoring and control activities for all mosquito species.
    c. Ovitraps will be serviced weekly, BG Sentinels weekly or daily at different stages of the experiment and the mosquitoes collected Eggs from ovitraps will be hatched under vacuum and the resulting larvae analysed. In that way, because the GM mosquitoes have an internal fluorescent marker and we examine the recaptured mosquitoes by microscopy- we are able to monitor the GM mosquito dispersal throughout the study site and beyond.

2) How (if) MRCU will track gene flow through the wild population?
    a. As above.

3) Size and location of target and control areas?

4) To confirm that Part 3 refers purely to *A. aegypti*. (Part 3 states that: "600,000 transgenic males would be released every week for a nine month period in West Bay. It is expected that the total number released would be around 22 million males".
    a. It does.

5) Details of concurrent operation that could be instigated if numbers of *A. albopictus* increase during the trial? (Part 8) For example, numbers of modified *A. albopictus* that would be released, that there is no difference in all potential environmental and human impacts of *A. albopictus* from *A. aegypti*, whether import permission for "an appropriate amount of *Aedes albopictus* eggs" will be obtained at the same time as permission to import the *A. aegypti* eggs is obtained, etc.
    a. The impacts of *A. albopictus* from *A. aegypti* are exactly the same. We seek import permission for the *Aedes albopictus* at this time pre-emptively in the minute chance that the are required. We cannot say specifically how many eggs would be imported at this time as it is on an as-needed basis, however it will far dwarf the numbers of *A. aegypti*.

Before deciding on the application MRCU is requested to consider amending the proposal to take in to account the following suggestions.

A) Host a community information session well in advance of the proposed project (at least two months), with community feedback becoming a part of the decision to pursue this project or not.

- Community Approval – Page 3, point 2 - "I [the applicant] will obtain community approval through contact with the Cayman Islands Department of Environment, who will assist in the further approach to the community if they feel the work is appropriate."

- The Department of Environment would be willing to facilitate this public meeting (but could not fund it) if MRCU would like to have it hosted by a third party.

B) Releasing to the public the project report mentioned in Part 6.

C) To be combined with an offer to speak with school science classes about the project, taking the opportunity to present novel science happening locally to the students.

Answer Received: MRCU has a thorough Public Engagement Strategy outlined. The Strategy is attached for consideration.

MRCU's Proposed Public Engagement Strategy for Release of OX513A Mosquitoes in an Area of Grand Cayman

Rationale: Though the reach of MRCU extends throughout the Cayman Islands, the release will only be conducted in a small area of West Bay. However, MRCU believes it essential that all who govern, work alongside, and are the proposed beneficiaries of its activities, be given appropriate time to comment on, and be updated on the progress of the release. The proposed strategy will deliver balanced information about the project to all stakeholders using appropriate means of communication.

Timeline: 30 days prior to commencement of project, and throughout the project

Stakeholders:

Government Level

- All cabinet members will be informed about the project via Briefing Note at Caucus

Civil Service and Relevant Authorities

- Details of the trial will be circulated throughout the Civil Service

Civil Society

- The project may be discussed with people that represent, and are advocates for the community if there is substantive interest

Community

- Information will be disseminated through media :
  - literature (pamphlets, posters, signage)
  - television (all-age accessible cartoon describing the science behind the trial with loveable characters Hades and Aegypta)
  - newspaper (print and online)
  - The community can make their voices heard via an opinion poll (print and online)
  - MRCU website (via "Research" tab publishing key details of the trial with FAQs and links to Oxitec website, and "Outreach" tab to publish photos and video of past events and schedules of upcoming events
  - social media (facebook, twitter)
  - public education (documented school visits)

Additional background provided by MRCU with their request to release genetically modified *Aedes aegypti* and *Aedes albopictus* mosquitos.

RIDL Technology: Further Questions

- As there is manual sorting of males and females, there is the possibility that some RIDL females may be released. In addition up to 5% of RIDL-wild type progeny will be bighting females. How is this managed?

The sorting is performed using a proprietary piece of equipment known as a wire sorter. This equipment sorts male and female pupae by size, males are smaller and can pass through the wire grid whereas females are larger and cannot. Quality assurance procedures in place during the sorting assure that no more than 1% females could be co-released. However in practice, efficiencies of sorting result much lower numbers of females (0.07% in Cayman in 2010 and 0.02% in Brazil) [1]. In the laboratory, under optimal conditions, the survival of functional adults is 5% or less. Evidence suggests that survival is much lower than this in the field; monitoring of ovitraps failed to detect any evidence of the transgene in eggs from the field two weeks after releases had stopped [1]. Additional studies investigating the characteristics of these low numbers of heterozygous survivors have also been carried out. When reared through immature stages at temperatures other than the ideal 27°C, the number of surviving adults is further reduced. Therefore although there is the potential for females to be released, these are unlikely to be fit and survive well in the environment.

- With the small likelihood that the population will be exposed to transgenic females, is there a study to determine whether the bite will cause allergenicity or adverse reaction in humans?

In order to present a risk to human health, the expressed proteins tTAV and DsRed2 would have to (a) be expressed in salivary glands, (b) be secreted into the saliva, and (c) be toxic or otherwise hazardous to humans if injected in relevant quantities. Of these, (a) and (b) relate to potential exposure, while (c) relates to the hazard incurred by the gene products. Addressing (c) first, Oxitec has commissioned extensive analysis on the safety of the expressed gene products using internationally recognized criteria, and it has been determined that there are no toxicological or allergenicity concerns from the gene products (tTAV and DsRed2) expressed in the OX513A Ae. aegypti. In addition tTAV expression is reported in a wide variety of organisms [2, 3] and a feeding study using OX513A [4] showed no adverse effects. Additional studies around the small numbers of survivors(above) assure us there is not only minimal risk of the strain establishing in the environment, but that any potential human exposure through the bite of a female would be very small.

- Is there information about how viruses will propagate in a RIDL mosquito, and what the interaction between virus and the transgenic components will be?

Vectorial competence is the intrinsic ability for the mosquito to become infected and subsequently transmit the pathogen [5]. No significant differences were detected in infection rates between OX513A strain and the comparative wild type strain for dengue serotypes 1-3 and chikungunya (manuscript in prep, available on request). The only significant difference detected was that the comparator strain females had a higher infection rate for dengue 4 than OX513A. Overall the impact of the transgene insertion had no effect on dengue or chikungunya susceptibility of the OX513A strain in this study.

- Is there a possibility of gene transfer to non-target species?

Vertical gene transfer refers to the ability of genes to move via sexual reproduction, to a closely related species or wild relative. *Ae. albopictus* (Skuse) and *Ae. aegypti* (L.) belong to different taxonomic divisions of the large subgenus *Stegomyia* and are native, respectively, to Asia and Africa. Both species however are invasive and can be found living side by side. *Ae. aegypti* mating is extremely species-specific. There have been reports of mating with the related species *Ae. albopictus* in both the laboratory and the field [6, 7], but even these matings, if successful, appear to fail to produce embryos capable of developing to adulthood. Forced laboratory matings have been conducted with OX513A and *Ae. albopictus* [8]; none of the eggs produced were fertile. Consequently vertical gene transfer to closely related mosquito species is both theoretically and practically possible but is likely to be an extremely rare event, and when these mating's occur naturally or are forced to occur as in close contact in a laboratory situation, fertile offspring are not produced. The strain OX513A

contains no toxic proteins or DNA that would confer a selective advantage to an organism; in fact the contrary is true as they confer a strong selective disadvantage. Consequently any hypothetically transferred genetic material would be rapidly lost from the recipient population. Reviews of the fate of DNA from transgenic organisms ingested by animals conclude there are no safety concerns that could be identified [9, 10]. As mentioned earlier studies have been conducted on non-target organisms consuming OX513A at 100% of diet and no adverse effects were noted.

- Given that global regulation on the use of GM insects has yet to be instated, what about the risk of transgenic eggs or even adults spreading to jurisdictions that have not approved their use, and are there any strategies to mitigate where this may be an issue?

The dispersal of OX513A adults by spontaneous flight, has been shown to be less than 100 meters [11] and the average distance travelled by wild adults is well-known to be less than 200 meters [11-25]. Therefore the risk of dispersal of adults is extremely low, especially from an island. Adults could move by passive transport further and so can eggs (in cars, plant pots, plastic containers etc.); however any offspring that inherit the OX513A gene would have a large selective disadvantage (a maximum of 5% survive) and would be rapidly lost from the recipient population. In addition, control of the *Aedes aegypti* population where the OX513A males are released (previously shown to take about 6 months to get over 80% reductions) would significantly reduce the risk of egg dispersal as far less eggs would be produced.

1. Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, Oviedo MN, Lacroix R, Naish N, Morrison NI *et al*: **Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes.** *Nat Biotechnol* 2012, 30(9):828-830.
2. Gill G, Ptashe M: **Negative effect of the transcriptional activator GAL4.** *Nature* 1988, **334(6184)**:721-724.
3. Gong P, Epton MJ, Fu G, Scaife S, Hiscox A, Condon KC, Condon GC, Morrison NI, Kelly DW, Dafa'alla T *et al*: **A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly.** *Nat Biotechnol* 2005, **23**(4):453-456.
4. Nordin O, Donald W, Ming WH, Ney TG, Mohamed KA, Halim NAA, Winskill P, Hadi AA, Lacroix R, Scaife S *et al*: **Oral ingestion of transgenic RIDL Ae. aegypti larvae has no negative effect on two predator *Toxorhynchites* species.** *PLoS One* 2013, **8(3)** e58805.
5. Kramer L, Ebel G: **Dynamics of flavivirus infection in mosquitoes.** *Adv Virus Res* 2003, **60**:187-232.).
6. Nasci RS, Hare CG, Willis FS: **Interspecific mating between Louisiana strains of *Aedes albopictus* and *Aedes aegypti* in the field and the laboratory.** *Journal of the American Mosquito Control Association* 1989, **5**:416-421.
7. Tripet F, Lounibos LP, Robbins D, Moran J, Nishimura N, Blosser EM: **Competitive Reduction by Satyrization? Evidence for Interspecific Mating in Nature and Asymmetric Reproductive Competition between Invasive Mosquito Vectors.** *Am J Trop Med Hyg* 2011, **85(2)**: 265-270.
8. Lee H, Aramu M, Nazni W, Selvi S, Vasan S: **No evidence for successful interspecific cross-mating of transgenic Aedes aegypti (L.) and wild type Aedes albopictus Skuse.** *Tropical biomedicine* 2009, **26(3)**::312-319.
9. Alexander T, Reuter T, Aulrich K, Sharma R, Okine E, Dixon W, McAllister TA: **A review of the detection and fate of novel plant molecules derived from biotechnology in livestock production.** *Anim Feed Sci Tech* 2007, **133**:31-62.
10. Flachowsky G, Schafft H, Meyer U: **Animal Feeding Studies for nutritional and safety assessments of feeds from genetically modified plants: A review.** *J Verbr Lebensm* 2012, **7**:179-194.
11. Lacroix R, McKemey A, Raduan N, Lim K-W, Ming WH, Ney TG, Siti Rahidah AA, Salman S, Subramaniam S, Nordin O *et al*: **Open Field Release of Genetically Engineered Sterile Male Aedes aegypti in Malaysia.** *PLoS One* 2012, *accepted.*
12. Getis A, Morrison AC, Gray K, Scott TW: **Characteristics of the spatial pattern of the dengue vector, *Aedes aegypti*, in Iquitos, Peru.** *Am J Trop Med Hyg* 2003, 69(5):494-505.
13. MacDonald PT: **Population characteristics of domestic Aedes aegypti (Diptera: Culicidae) in villages on the Kenya coast. II. Dispersal within and between villages.** *Journal of Medical Entomology* 1977, **14**:49-53.
14. Maciel-de-Freitas R, Lourenco-de-Oliveira R: **Presumed unconstrained dispersal of Aedes aegypti in the city of Rio de Janeiro, Brazil.** *Rev Saude Publica* 2009, **43**(1):8-12.

15. Ordonez-Gonzalez JG, Mercado-Hernandez R, Flores-Suarez AE, Fernandez-Salas I: **The use of sticky ovitraps to estimate dispersal of Aedes aegypti in northeastern Mexico.** *J Am Mosq Control Assoc* 2001, **17**(2):93-97.
16. Reiter P: **Oviposition and dispersion of Aedes aegypti in an urban environment.** *Bulletin de la Societe de pathologie exotique (1990)* 1996, **89**(2):120-122.
17. Reuben R, Panicker KN, Brooks GD, Ansari MA: **Studies on dispersal and loss rates of male *Aedes aegypti* (L.) in different seasons at Sonepat, India.** *WHO/VBC* 1975, **531**.
18. Russell RC, Webb CE, Williams CR, Ritchie SA: **Mark-Release-Recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia.** *Medical and Veterinary Entomology* 2005, **19**:451-457.
19. Tsuda Y, Takagi M, Wang S, Wang Z, Tang L: **Movement of Aedes aegypti (Diptera: Culicidae) released in a small isolated village on Hainan Island, China.** *J Med Entomol* 2001, **38**(1):93-98.
20. Harrington L, Scott TW, Lerdthusnee K, Coleman RC, Costero A, Clark GG, Jones JJ, Kitthawee S, Kittayapong P, Sithiprasasna R *et al*: **Dispersal of the dengue vector *Aedes aegypti* within and between rural communities.** *Am J Trop Med Hyg* 2005, **72**(2):209-220.
21. Reiter P, Amador MA, Anderson RA, Clark GG: **Short report: dispersal of Aedes aegypti in an urban area after blood feeding as demonstrated by rubidium-marked eggs.** *Am J Trop Med Hyg* 1995, **52**(2):177-179.
22. Trpis M, Häusermann W: **Dispersal and Other Population Parameter of *Aedes aegypti* in an African Village and their Possible Significance in Epidemiology of Vector-Borne Diseases. .** *American Journal of Tropical Medicine and Hygiene* 1986, **58**:1263-1279.
23. Maciel-de-Freitas R, Torres Codeco C, Lourenco de Oliviera R: **Body size associated survival and dispersal rates of *Aedes aegytpi* in Rio de Janerio.** *Medical and Veterinary Entomology* 2007, **21**(3):284-299.
24. Maciel- de- Freitas R, Torres Codeço C, Lourenço-de-Oliveira R: **Daily Survival Rates and Dispersal of *Aedes aegypti* females in Rio de Janeiro, Brazil. .** *The American Journal of Tropical Medicine and Hygiene* 2007, **76**(4):659-665.
25. Muir L, Kay B: ***Aedes aegypti* survival and dispersal estimated by mark-release –recapture in Northern Australia** *Am J Trop Med Hyg* 1998, **58**::277-282.