Risk analysis – OX513A *Aedes aegypti* mosquito for potential release on the Cayman Islands (Grand Cayman).

Prepared – Oct 2009

Based on requirements for a Pest Risk Analysis (IPPC) and the Annex III Risk Assessment for the Cartagena Biosafety Protocol

SECTION 1. THE ORGANISM

1. **Name and taxonomic position (including any taxonomic subdivisions, difficulties or confusion: subspecies, pathotypes, *formae speciales*, overlapping species, synonymy).**

The recipient organism is the *Aedes (Stegomyia) aegypti* (L.) mosquito which belongs to the genus Diptera: Culicidae.. *Ae.aegypti* is the principle vector of dengue fever and dengue haemorrhagic fever.

The strain OX513A has been genetically modified to express a marker gene (red fluorescence) and a repressible lethal trait, resulting in a phenotype where male *Aedes aegypti* released with the genetic construct will pass on the lethal trait to both male and female offspring, causing them to die as larvae or pupae. No toxic proteins, plasmid backbone sequences, antibiotic resistance genes, insecticide genes or bacterial origins of replication have been introduced into the OX513A strain of mosquitoes.

Donor organisms that provided DNA for the genetic construct inserted into *Aedes aegypti* are detailed in Table 1 with a description of the genetic elements. Further information is given here:

Trichoplusia ni (Hűbner)(cabbage looper moth) a member of the *Lepidoptera: Noctuidae* is a leaf feeding pest of Crucifer plants where it causes economic losses, but will also feed on legumes, tomatoes and cotton.

Drosophila melanogaster (Meigen) a member of the Diptera family. It is known as common fruit fly or vinegar fly.

Discosoma sp are coloured marine coral. They are widespread in the marine environment and there have been many transgenic field releases where it has been used as a marker gene (nine releases that contained the DsRed gene or variants of it are recorded in the USA database at (http://www.isb.vt.edu/CFDOCS/fieldtests3.cfm)).

Escherichia coli is a bacterium that is widespread in the environment and the human GI tract. Some strains can cause illness, occasionally severe, but there are also benign laboratory strains that are used in the construction of plasmids for genetic transformation.

Herpes simplex virus type 1 is a human virus usually associated with infections of the lips, mouth, and face. It is the most common herpes simplex virus and many people develop it in childhood. HSV-1 often causes sores (lesions) inside the mouth, such as cold sores (fever blisters), or infection of the eye (especially the conjunctiva and cornea). It can also lead to infection of the lining of the brain (meningoencephalitis). It is transmitted by contact with infected saliva. By adulthood, 30 - 90% of people will have antibodies to HSV-1.

(a) Vector. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range;

The vector used is piggyBac, as DNA (deoxyribonucleic acid) transposons isolated from the Cabbage Looper moth, *Trichoplusia ni* that only when it's inverted terminal repeats (ITRs) are intact, is capable of integrating DNA flanked by an open reading frame (ORF) within the element (Handler et al , 2002; Handler et al 1998). In the construct used for transformation of the mosquitoes the transposase gene of the piggyBac element was irreversibly destroyed by deletion of a section of that gene. Transformation is effected by introducing with the transforming construct a helper plasmid that supplies transposase activity but is itself unable to transpose into other DNA. One of ITR's that flank the wild type piggyBac transposase has been removed in the helper plasmid so that the helper plasmid cannot, itself integrate, even though it encodes for the active transposase. The helper plasmid is not present in the modified mosquitoes.

It has been hypothesised (Wimmer 2003; Wimmer 2005) that the use of non-autonomous transposons to mediate insect transformation could lead to the risk of horizontal gene transfer to other organisms. Horizontal gene transfer (HGT), that is the movement of genes from multicellular (eukaryotic) organisms, such as insects, to bacteria is remarkably rare, occasionally being detected under optimized laboratory conditions, but never in natural or field conditions. HGT between eukaryotes has never been observed.

Non-autonomous transposon vectors that assist transformation of insects, such as the case of OX513A, have shown to be very stable following insertion into the *Aedes aegypti* genome and exposure to exogenous transposase under a wide variety of conditions (O'Brochta et al; 2003 : Sethuraman et al 2007).

Exchange of genetic material between insects of different species in the wild rarely happens as insects exchange gametes internally and have complex mating behaviours and structures. Even in laboratory conditions they cannot form fertile hybrids. These mating barriers restrict any introduced genes to species that contains them. Mosquitoes do not transfer any DNA whilst blood feeding eliminating any chance of gene transfer.

If, in the extremely unlikely event the organism does acquire a gene through horizontal gene transfer then it must maintain it to have an impact or consequence in the environment. To have an impact, a significant number of organisms must acquire the new gene. This depends on the rate of HGT, the nature of the gene, the incorporation of the gene into heritable cells and environmental influences. If maintaining the new gene has no advantage for an organism, the gene will not persist. Strain OX513A contains no DNA that would confer a selective advantage to another 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.

(b) **Insert or inserts and/or characteristics of modification. Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced**;

The inserted genetic elements and their function are detailed in Table 1.

Table 1: Genetic elements inserted into OX513A *Aedes aegypti* mosquitoes

Genetic Element	Donor Organism and Common name	Reference	Function	Comments
piggyBac 3'	*Trichoplusia ni* (Cabbage looper moth)	Cary et al Virology 1989 Sep: 172(1): 156-159 Thibault et al 1999	DNA transposable element with sequence deletions to prevent mobility.	See description under section c on Vector.
Act5C	*Drosophila melanogaster* (Vinegar fly)		Promoter element driving the expression of the marker gene	
DsRed2	*Discosoma (*Coral)	Ip and Wan (2004) Luminescence (Chichester) 19(3): 148	Red fluorescent protein marker gene	The Fluorescent marker has been used in a wide range of vertebrate and invertebrate species as marker genes, confer no competitive advantage of disadvantage to the recipient and no adverse ecological or other consequences resulting from their incorporation into the mosquito.

Drosomycin 3' UTR	*Drosophila melanogaster* (Vinegar fly)		Terminator region (polyadenylation signal)	
$TetO_7$	*Escherichia coli* (bacteria)	Gossen M and Bujard H (1992) Proc Natl Acad Sci USA (89(12): 5547-5551	Non-coding binding site for tTAV element	
Hsp70	*Drosophila sp.* (vinegar fly)		Promoter element driving tTAV element	
Adh intron	*Drosophila sp.* (vinegar fly)		Enhances gene expression	
tTAV	Synthetic DNA based on a fusion of sequences from *Escherichia coli* and Herpes simplex virus (VP16 transcriptional activator)	Gossen M and Bujard H (1992) Proc Natl Acad Sci USA (89(12): 5547-5551 Gong et al (2005)	Tetracycline repressible transcriptional activator	tTA protein binds to and activates the expression from the tetracycline response element (tRE) which includes the specific DNA sequence to which tTA binds (tetO). tTA aslp binds tetracycline with a high affinity, which then cannot bind DNA. In effect tTA acts as tetracycline regulated switch. High level expression of tTA is

				deleterious to cells as it can repress their normal transcription. tTA has been used in fungi, mice, plants, mammalian cultures with no known adverse effects on the environment or human health
3'UTR	*Drosophila sp*. (vinegar fly)		Terminator region	
piggyBac 5'	*Trichoplusia ni* (Cabbage looper moth)	Cary et al Virology 1989 Sep: 172(1): 156-159	DNA transposable element with sequence deletions to prevent mobility.	

(c) Living modified organism. Identity of the living modified organism, and the differences between the biological characteristics of the living modified organism and those of the recipient organism or parental organisms;

Two proteins have been introduced into the mosquitoes. The first is a fluorescent marker gene, DsRed2 that allows the modified mosquitoes to be fluorescent when excited by illumination of a specific wavelength (excitation wavelength 558nm, emission583nm). Expression of the fluorescent protein, DsRed2 will therefore allow the detection of the modified mosquitoes.

The second is the protein tTAV, which is deleterious to the insect when expressed in sufficient quantity to repress normal cell transcription in the absence of tetracycline. In the modified mosquitoes, the protein is under the transcriptional control of tRE and is therefore expressed when tTAV is present and tetracycline is not. Basal expression of tTAV in the modified mosquitoes has no effect on the modified mosquitoes when reared under normal conditions in the presence of tetracycline. High level expression of tTAV in the absence of tetracycline is deleterious to the insect, leading to insect death (Fu et al, 2007). This tetracycline regulated system has been widely used (Fussenegger, 2001, Koukidou et al, 2006). The use of the

repressible system has beneficial consequences in that the system is activated when insects are released into the environment. This provides a "fail-safe" lethal mitigation to the release of autocidal OX513A strains. A potential hazard arising from dead insects persisting in the environment is highly unlikely because the dead material will contain no toxic compounds and consists of ubiquitous proteins, nucleic acids, carbohydrates, minerals, fats and other organic compounds.

(d)Vector. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range;

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Non-autonomous transposon vectors that assist transformation of insects, such as the case of OX513A, have shown to be very stable following insertion into the *Aedes aegypti* genome and exposure to exogenous transposase under a wide variety of conditions (O'Brochta et al; 2003 : Sethuraman et al 2007).

Exchange of genetic material between insects of different species in the wild rarely happens as insects exchange gametes internally and have complex mating behaviours and structures. Even in laboratory conditions they cannot form fertile hybrids. These mating barriers restrict any introduced genes to species that contains them. Mosquitoes do not transfer any DNA whilst blood feeding eliminating any chance of gene transfer.

2. Relationship with known quarantine pests.

Aedes aegypti is not a quarantine pest but is a significant vector of dengue fever and other arboviruses. Significant effort is underway worldwide for the control of the vector. It is an invasive species, originating in Africa but achieving pan tropical distribution in the 1930's. It is not a native species in the Cayman Islands but is currently present on Grand Cayman (see item 2.2).

3. Methods for identification for inspection purposes.

Ae. aegypti is a medium-sized blackish mosquito easily recognized by a silvery-white 'lyre-shaped" pattern of scales on its scutum. Segments 1 to 4 of the hind tarsi possess broad basal white rings, segment 5 is white. The coloration of both sexes is similar.

The modified mosquitoes (OX513A) can easily be identified as larval and pupal life stages will be fluorescent under illumination of specific light wavelengths.

4. Methods for detection.

There are a variety of detection methods; trapping methods, fluorescence methods and molecular biology methods.

Trapping methods:

The trapping methods employed may consist of both ovitraps which can trap eggs/larvae and those traps that are used for collection of adults. Each will be briefly described:

Ovitraps are a sensitive method for assessing of presence at low population levels, and can also give an indirect measure of adult abundance. There are low tech/cheap, easily deployed so can be deployed in relatively high numbers allowing spatial and temporal assessments. Variants of the ovitraps, known as sticky ovitraps can also catch gravid female adults. They do suffer some drawbacks: Maintenance and servicing is labour intensive. Ovitraps cannot be used as accurate measure of adult population due to high variability in the survival of eggs and successive developmental stages to adult. Ovitrap data is often cited in the form of the Ovitrap index:

- **Ovitrap Index (OI)** =

(Number of positive ovitraps / total number inspected) x 100

Ovitraps can be used to assess the wild mosquito population in advance of the trial

Adult collection methods

Adult recapture can be conducted using BG-Sentinel traps. The BG-Sentinel trap is a relatively newly developed trap specifically targeting *Aedes* mosquitoes. These traps have been extensively field tested in direct comparison with other live adult sampling methods including the CDC backpack aspirator, human bait landing catch, and Fay-Prince traps (Maciel de Freitas, 2007). BG-Sentinel traps generally outperform other types of traps, and capture comparable or significantly more *Aedes aegypti* over 24 h, compared to a standard CDC backpack aspirator household survey (15-30 min).

Unlike aspiration surveys, BG-Sentinel traps catches will be independent of operator skill, are less intrusive that some other sampling methods, and less labour intensive. The negative is that they are relatively expensive (~$300) and need to be connected to mains power or a heavy-duty batteries (small car/motorbike) to operate the fan.

Fluorescence and molecular biology methods:

Fluorescence-based detection: The transgenic construct encodes a fluorescent protein (DsRed) which allows LMO larvae and pupae to be accurately discriminated from wild type. This marker is extremely reliable. At least 500,000 LMO larvae and pupae have been screened for fluorescence with no false negatives and at least 1,000,000 with no false positives.

DNA sequence-based detection: Using the polymerase chain reaction (PCR) or similar methods, specific DNA sequences can be detected with extremely high sensitivity and accuracy; in principle from a single cell, or from old or degraded specimens. Indeed such methods are widely used in criminal forensics because of these attributes of sensitivity and accuracy. Since the LMO contains exogenous DNA sequences not normally found in *Aedes aegypti* (e.g. from *Trichoplusia ni*, *Discosoma*, etc), the LMO can readily be distinguished from wild type by this method.

SECTION 2. BIOLOGICAL CHARACTERISTICS OF THE PEST

1. Life cycle:

(*a*) *for invertebrates*

rate of development (typical times, or degree days, for successive life-cycle stages; reproduction rate);

The eggs are laid individually by females in the damp walls of both natural and artificial containers that can hold water. Eggs are the long- term survival structures of these mosquitoes, surviving 6 months to a year depending on the conditions. The larvae and pupae prefer relatively clean water typically found in types of container: water storage containers, flowerpots and waste materials such as tyres, cans, bottles etc. The waste material containers are usually only sources of mosquitoes during the rainy season in other countries but in tropical countries this tends to be year round.

The characteristics of the OX513A *Aedes aegypti* have been thoroughly evaluated by several institutions worldwide, e.g. in France, Malaysia (Lee et al, 2008) and Thailand (Khongtak et al , 2009). The studies in Thailand and Malaysia were carried out at Arthropod Containment Level -2. The IMR has evaluated the characteristics of the OX513A strain in a simulated semi-field environment, using a field house that replicated at typical Malaysian dwelling. The field house is large enough to virtually eliminate chance/ forced matings encounters and improves the reliability of mating competitiveness experiments over small cages. It has been found by IMR that there were no differences between the OX513A strain and the wild-type for the numbers of eggs laid, number of unhatched eggs, egg hatching rate, number of hatched larvae, duration of larval period, larval survivorship, pupation, adult eclosion rate, gonotrophic cycle, adult fecundity and offspring ration (Lee et al, 2009b)

number of generations per year (univoltine, multivoltine, how voltinism is controlled);

Number of generations per year is temperature dependant but is approximately 12/year.). Females mate only once in their lifetime and need a bloodmeal (ie bite humans) to allow the eggs to develop.

hosts

Humans are the primary hosts of *Aedes aegypti*, an extremely anthropophilic mosquito. The preferred sites for adults are domestic urban environments in sheltered dark spaces within houses/ apartments. *Aedes aegypti* is a day biting mosquito with two peaks, one mid-morning and one mid-afternoon.

parthenogenetic multiplication;

Aedes aegypti does not have parthenogenetic multiplication.

typical timing of the life cycle

Generation time is dependent on temperature and humidity; eggs hatch within a week if conditions are favourable, larval development is around 5-10 days with the pupation stage taking approximately 2-3 days. Under the most favourable conditions egg to pupae can take as little as 7 days. Adults can survive from 2 weeks to over 1 month. Males tend to live around 7 days if females are present, but can survive longer without the presence of females. . The average adult lifespan is 8-15 days for female mosquitoes and 3-6 days for male mosquitoes. Average generation time is approximately 1 month.

The fitness of the OX513A strain and wild strain of *Aedes aegypti* has been shown to be not significantly different in several parameters (Lee et al, 2008) There was no significant difference in numbers of eggs laid, number of larvae hatched and the number of pupae in F1 generations, nor in the number of days in each stage of life (developmental period). The number of larvae, pupae surviving and adult emerged in each stages of life for both strains were not significantly different. Even though no fitness advantage has been identified in the OX513A strain over the comparator strain, any kind of fitness advantage that might conceivably exist would have to compete for survival against overwhelming reproductive sterility.

2. Dissemination and dispersal:

natural means, speed and range of dissemination;

Spontaneous flight of adults is limited to around 200m depending on availability of breeding sites, and hosts from which to take a blood meal. However the species is dispersed by passive transport on boats, trains etc and International Sanitary Regulations (WHO, 2005) require ports and airports to be clear of *Ae.aegypti* for 400m. Climate and the availability of breeding sites are the two main factors that regulate the populations of *Ae.aegypti* in urban environments

vectors: occurrence of known natural vectors or related species with vector potential

Aedes aegypti was eliminated from the island in 1971 but since then there have been numerous introductions onto Grand Cayman in 1973, 1980 and 1991-96, which were

eliminated (Petrie et al, 2007). In 2002 *Ae.aegypti* was detected on Cayman and remains present (Wheeler and Petrie, 2007), and it is the intended effect of the release for the OX513A male mosquitoes to exchange genetic material with the females Aedes aegypti present on the island.

The related species *Aedes albopictus*, a secondary vector of dengue was detected on Grand Cayman in 1997, one year after *Aedes aegypti* was eradicated. In 2007 the levels of *Aedes albopictus* were accounting for approximately 5% of the lavarval finds on the island (Wheeler and Petrie, 2007).

Exchange of genetic material between insects of different species in the wild rarely happens as insects exchange gametes internally and have complex mating behaviours and structures. Mosquitoes are not capable of interbreeding with other insect species. So each mosquito species can interbreed only with its own species, and not with other species. Even in laboratory conditions they cannot form fertile hybrids. These mating barriers restrict any introduced genes to species that contains them.

Mosquitoes do not transfer any DNA whilst blood feeding eliminating any chance of gene transfer.

OX513A mosquitoes have been modified to include dominant lethal mutations within their genetic structure (Alphey et al, 2002). The repressible lethality element is a self mitigating trait in the environment, as the progeny that inherit the genetic element will die, without access to the levels of dietary supplement that is required to repress the lethal element. Such genes do not confer a selective advantage to the insect; quite the opposite, they confer a very strong *dis*advantage. If maintaining the new gene has no advantage for an organism, the gene will not persist. Consequently any hypothetically transferred genetic material would be rapidly lost rather than spread, from any non-target population that might be exposed to them, such as *Aedes* mosquitoes in areas immediately adjacent to any release area.

3. Survival of adverse conditions:

(*a*) *for invertebrates*

capability for winter or summer diapause and relevant climatic cues; physiological adaptations for survival of low temperatures, desiccation etc. in or out of diapause;

The strain to be used is a non-diapausing strain. Eggs are the survival form of *Aedes aegypti* and can survive without water for 6months – 1 year depending on the conditions. Hatching is effected by availability of water.

SECTION 3. GEOGRAPHICAL DISTRIBUTION OF THE PEST

1. Present occurrence in PRA area.

Aedes aegypti is not a native species to the Cayman Islands, and is an introduced species. It was eliminated from Grand Cayman in 1971 but since then there has been numerous introductions onto Grand Cayman in 1973, 1980 and 1991-96, which were eliminated (Petrie et al, 2007). The sister islands of Little Cayman and Cayman Brac remain free of *Aedes*

aegypti. In 2002 *Ae.aegypti* was detected on Grand Cayman and remains present (Wheeler and Petrie, 2007), and it is the intended effect of the proposed release for the OX513A male mosquitoes to exchange genetic material with the females.

The proposed trial site of East End on Grand Cayman is a candidate trial site as it meets the following criteria:

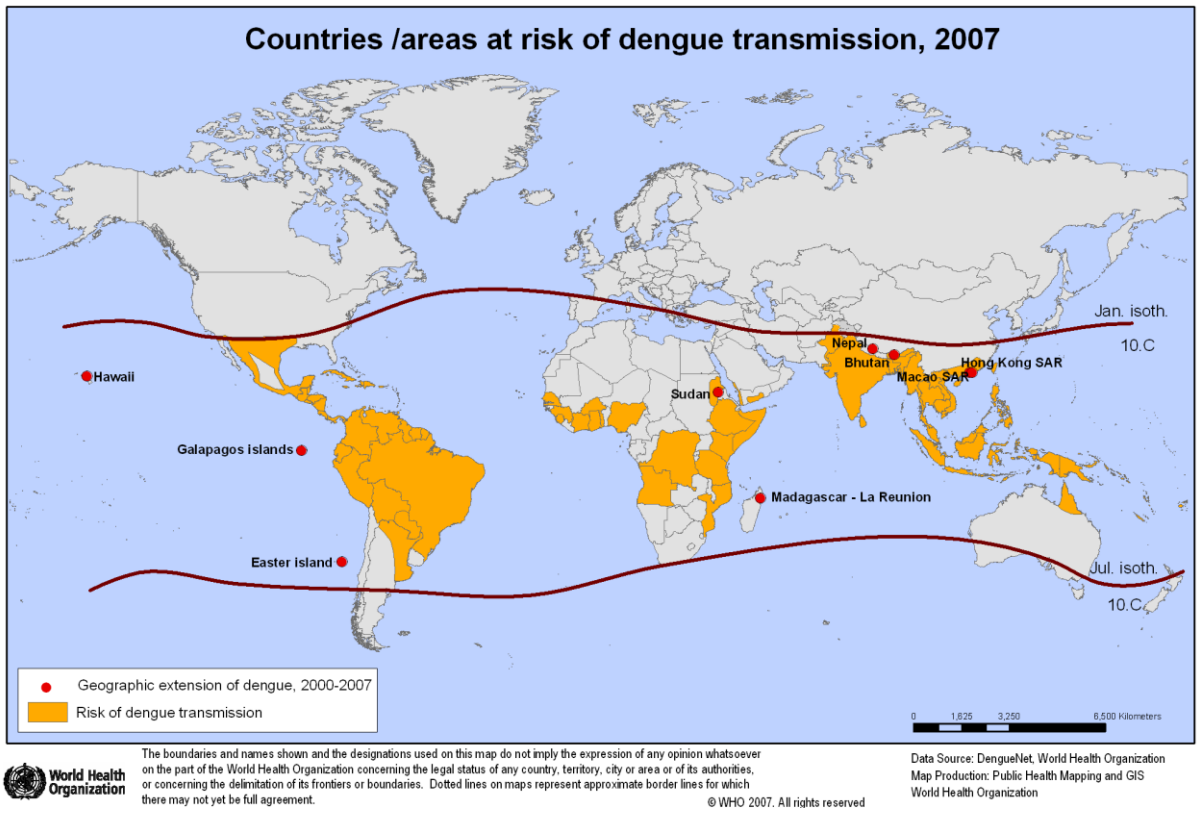
a) Inhabited by humans
b) Dominate presence of *Aedes aegypti*
c) Reasonable accessibility with infrastructure for establishing a field station
d) Co-operation and support of local population and health authorities
e) Ecologically stable
f) Geographically isolated to limit the immigration of mosquitoes from neighbouring communities.
g) Presence of comparable sites which can act as untreated control.

Not all these criteria are required for a limited release study; however it would be beneficial to conduct limited release studies in the same area as further interventions such as suppression studies. The proposed trial site has approximately 2-3,000 people, and a comparable control site is Bodden Town, approximately 7km to the west.

Further details concerning the proposed release are detailed in the protocol document (separate to this document).

2. World distribution (map if possible), by countries and areas within countries or by region or continent (e.g. West Africa) depending on information available, with indication if possible on status of each record (confirmed or not, old or new, pest established or not).

DengueNet, built on the Global Health Atlas platform, is the World Health Organization's central data management system for the global epidemiological and virological surveillance of dengue fever (DF) and dengue haemorrhagic fever (DHF) created in partnership with WHO Regional and Country Offices, Ministries of Heath, WHO Collaborating Centres and laboratories. The network collects standardized data from all DengueNet partners worldwide, and provides ready access to a variety of indicators such as incidence, case fatality rates (CFR), frequency and distribution of DF and DHF cases, number of fatalities and circulating virus serotypes. All countries with confirmed cases of dengue fever and related conditions will contain the vector, *Aedes aegypti*. DengueNet is available online and can be queried at various levels (http://apps.who.int/globalatlas/default.asp).



3. Area of origin and history of any spread from area of origin.

Ae.aegypti is a tropical species of mosquito found between 15°N and 15°S, typically in Africa and parts of South America (WHO). It originated in Africa and achieved pan-Tropical distribution in the 1930's. See map above for the current distribution.

SECTION 5. POTENTIAL OF THE PEST FOR ESTABLISHMENT IN PRA AREA

1. Ecoclimatic zones of the pest's distribution comparable with those found in PRA area

See section 3.2 above. Grand Cayman is within the zone for the risk of dengue transmission and there have been six confirmed cases of dengue on the island in Oct –Nov 2007 (Wheeler and Petrie, 2007) as a result of residents returning with dengue from elsewhere. There were no reported cases of local transmission in 2007.

2. Climatic conditions (e.g. temperature, rainfall, RH, day length) which have been shown to be conducive or suppressive to survival, development, reproduction and dispersal of the

pest (where such conditions are not explicitly known, infer as far as possible what elements in the pest's geographical distribution gives clues of these conditions).

Cayman has a subhumid tropical climate with distinct seasonal variation. There are no other large land masses within a 200km radius and the climate is strongly moderated by the sea (Burton, 1994). The wet season occurs from May through November with an average maximum daily temperature of around 28^{o}C. The dry season occurs December –April with the average maximum daily temperature of 24 -25^{o}C. Average rainfall ranges from 1,107mm in the east of the island through to 1,595mm in the western portion. Flooding can also be an issue after heavy rains. Although weather patterns are generally stable, the island is subject to low pressure systems, forming tropical depressions, storms and hurricanes in the summer months. The last major hurricane that hit Grand Cayman was Hurricane Ivan in 2004.

Climate, particularly temperature and the availability of breeding sites are the two main factors that regulate the populations of *Ae.aegypti* in urban environments.

SECTION 6. CONTROL OF THE PEST

1. **Control measures in regular use in any part of the pest's geographical range, particularly in areas where the climate is comparable to that of PRA area:**
   a) current control measures using plant protection products, together with an estimate of their efficacy;

The Mosquito Research and Control Uses (MRCU) on Grand Cayman use three components for the control approach for *Aedes aegypti*: prevention of entry (Port Disinsection), ovipot surveillance and chemical controls. Applications of insecticides are against both adult and larval stages, but restricted to outdoor breeding sites for the detection of larvae. Insecticides include methoprene, temephos and *Bacillus thuringiensis* var. *israelensis* (Bti). Based on the results, yards found to be consistently positive for breeding are routinely treated with residual wall or barrier treatment with permethrin, temephos, deltamethrin, lambdacyhalothrin and bifenthrin. The effectiveness of current methods is hard to quantify but it can be concluded that they are not 100% effective due to the continued re-introduction of *Aedes aegypti* into Grand Cayman (Wheeler and Petrie, 2007)

b) evidence of resistance to plant protection products;

This work has been conducted by MRCU. The OX513A strain has been assessed for insecticide resistance to current control insecticides and no significant resistance was detected from bioassays or molecular analysis.

c) cultural or other control measures not using plant protection products;

Owners/occupiers are required by law (Mosquito Research and Control Law, 2007) to trim trees, shrubs, plants, and rank grass from their premises to discourage mosquito resting sites and increase the efficiency of control measures. Owners/occupiers are also required by the same law to keep premises clear of potential breeding sites such as tyres, tins, bottles and other articles likely to collect water, as well as mosquito-proof all inlets and outlets of water cisterns, septic tanks and pit latrines.

d) possibilities for treatment of consignments against the pest.

Port disinsection is used in Cayman on all vessels arriving from overseas (Wheeler and Petrie, 2007)

2. Records of eradication (successful or attempted): methods used;

Cayman Islands have had several successful eradication programmes for *Aedes aegypti*, using temephos insecticide (Nathan and Giglioli, 1982). House to house inspections, population assessments and insecticidal treatments are all essential for a successful eradication program.

SECTION 7. TRANSPORT OF THE PEST

1. Method of natural spread elsewhere in the world (cf. 2.2).

Human associated transport activities are the key means of spread of *Aedes aegypti.* Aircraft, ocean going vessels and the increase in the movement of containerised goods, despite port disinsection all contribute to the world wide dissemination of *Aedes aegypti.*

SECTION 8. INFORMATION RELATING TO THE INTENDED USE

The intended use of OX513A strain is intended to be used to suppress the target population in a method analogous to the Sterile Insect Technique (SIT). The idea of releasing sterile insects in large numbers to control pests is itself not new, but a well-known concept that was developed by American entomologists Raymond Bushland and Edward Knipling in the 1950s for which they received the 1992 *World Food Prize* (Dyck et al. 2005; Knipling 1955). The Sterile Insect Technique (SIT) has set very successful precedents around the world, most notably by controlling the Mediterranean fruit fly or medfly (*Ceratitis capitata*), and by eradicating the new world screw worm (*Cochliomyia hominivorax*) from the United States and Central America all the way down to Panama.

However, classical SIT has hitherto not been successful with mosquitoes such as *Aedes* in spite of much effort by the International Atomic Energy Agency (IAEA) and others because gamma radiation, used in classical SIT to sterilise the insects, renders the mosquitoes very weak and unfit to compete with the wild male mosquitoes. However, this problem seems to have been overcome because OX513A uses genetic methods instead of radiation to achieve sterility, therefore the genetically sterile insects have been reported to be fitter and competitive (Jain 2006; Lee et al. 2006; Phuc et al. 2007;).

The genetically sterile (homozygous) male *Aedes aegypti* mosquitoes which mate with wild females to produce (heterozygous) offspring that will all die as larvae or pupae in the absence of a food supplement (Phuc et al. 2007).

The fitness of the OX513A strain and wild strain of *Aedes aegypti* has been shown to be not significantly different in several parameters (Lee et al, 2008) There was no significant difference in numbers of eggs laid, number of larvae hatched and the number of pupae in F1 generations, nor in

the number of days in each stage of life (developmental period). The number of larvae, pupae surviving and adult emerged in each stages of life for both strains were not significantly different.

In the case of either an inadvertent release of OX513A mosquitoes into the environment (due to unforeseen circumstances, accidents, natural disasters, etc.), there is an in-built genetic containment (fail safe) mechanism within the introduced genetic system that would prevent the strain from establishing itself in the wild.

For example, following a release of OX513A males, then any wild type females that mate with these males would result in F1 progeny that are heterozygous for the autocidal trait and are thus 'pre-destined to die' as there is an insufficient dose of the dietary antidote, tetracycline, in the environment. If there is an unintended release of OX513A females, then any of the following three things could happen: (i) these OX513A females mate with a wild type male and produce F1 progeny, or (i) these OX513A females mate with a OX513A male and produce F1 progeny, or (iii) these OX513A females die due to predation or natural causes without producing F1 progeny. The third case poses no risk at all, while the first and second cases will respectively result in heterozygous or homozygous autocidal F1 progeny that are, once again, 'pre-destined to die' due to lack of tetracycline in the environment. Thus, we see that these mosquitoes cannot establish themselves in the environment either from an inadvertent release of both males and females of the RIDL OX513A strain or from a deliberate release of OX513A males.

The adverse environmental consequences of using OX513A male sterile mosquitoes whose offspring die are likely therefore to be of extremely limited consequence due to the self limiting nature of the proposed release.

SECTION 9. INFORMATION ON ENVIRONMENTAL INTERACTIONS

1. **A description of the receiving environment**

   Aedes aegypti is a domestic/ peri-domestic mosquito and preferentially lives in human habitations, shops etc. *Aedes aegypti* prefers dark resting places inside houses and buildings. Therefore the target ecosystem is urban and likely to contain, but not exclusively other mosquito species, spiders, reptiles and amphibians, and small domestic animals such as cats and dogs, rabbits etc. Insectivorous birds, bats and/or fish could also predate on mosquitoes.
   The biodiversity of the urban and man-made areas has been collated in the Cayman Islands National Biodiversity Action Plan (NABP, 2008), which reported there were no protected areas in the urban and man-made habitat category. Key species identified in the NABP 2008 for urban areas, that could be potential non-target organisms included: all bats (Chiroptera); all birds, specifically Grand Cayman Parrot (*Amazona leucocephala caymanensis*), Indian Whistling duck (*Dendrocygna arborea*), and Great Antillean grackle (*Quiscalus niger caymanensis*), white winged dove (*Zenedia asiatica*), reptiles; Eastern grand Cayman blue throated anole (*Anolis conspersus lewisi*), Grand Cayman racer (*Alsophis cantherigerus caymanus*), Grand Cayman water snake (*Tretanorhinius variabilis lewisi*), Grand Cayman

ground boa (*Tropidphis caymenensis caymenensis*), Grand Cayman blind snake (*Typhlops caymenensis*), Taco river slider (*Trachemys decussata angusta*).

Grand Cayman is located at 19°18'N, 81°16'W, the largest of the three Cayman Islands. Grand Cayman is 35km long and is orientated East-West. The centre of the island is swamp land (over 50% of the island) with mangrove swamps and seagrass beds. The major population centres are situated within the peninsular region on the Western side of the island; however there are towns on the southern and eastern side.

2. Potential interactions with non –target species

a) Bats

There are nine native species of bats on the Cayman Islands. Each of these specializes in a different type of food and each has a different role in the ecosystem. The frugivore and pollen eating bats are not listed as they do not eat insects, leaving six species of insectivore bats. However bats are largely nocturnal and *Aedes aegypti* is a mosquito whose activity has two daily peaks (sunrise and sunset) and therefore it is likely that bats will have limited interactions with the OX513A mosquitoes.

Common Name	Classification	Diet	Status	Distribution
Family: Phyllostomidae				
Subfamily: Phyllostomatinae				
Big-eared Bat	*Macrotus waterhousii minor*	Insectivore	Rare	Bahamas, Cayman Islands, Cuba
Family: Vespertilionidae				
Big Brown Bat	*Eptescus fuscus dutertreus*	Insectivore	Rare	Cayman Brac, Cuba
Brown Bat	*Eptescus fuscus spp. nova*	Insectivore	Very Rare	Grand Cayman
Red Bat	*Lasiurius spp unconfirmed*	Insectivore	Very Rare	Unconfirmed
Family: Mollossida				
Brazilian Free-tailed Bat	*Tadarida brasiliensis muscala*	Insectivore	Rare	Cuba, Grand Cayman
Velvety Free-tailed Bat	*Molossus molossus tropidorhynchus*	Insectivore	Common	Cayman Brac, Cuba, Grand Cayman

Source Cayman Wildlife (caymanwildlife.org)

b) Birds

Endemic landraces of birds on Cayman Islands are listed below; these birds have limited geographic range and are endemic to Cayman Islands. Again only the insectivorous feeders are listed.

	The endemic races of landbirds found on Grand Cayman (GC), Little Cayman (LC) and Cayman Brac (CB)			
Common Name	**Classification**			**Status**
Grand Cayman Thrush	*Turdus ravidus*	recent on GC	Diet	Extinct
W. Indian Woodpecker	*Melanerpes superciliaris caymanensis*	GC	Fruit and insects (Cruz and Johnstone 1984)	
Northern Flicker	*Colaptes auratus gundlachi*	GC	Insects fruit, seeds and nuts	
Caribbean Elaenia	*Elaenia martinica caymanensis*	GC, LC, CB	Insects and fruit	
Loggerhead Kingbird	*Tyrannus caudifasciatus caymanensis*	GC, CB	Insects and fruit	
Thick-billed Vireo	*Vireo crassirostris alleni*	GC, CB	Insects and fruit	
Yucatan Vireo	*Vireo magister caymanensis*	GC	Insects and fruit	
Vitelline Warbler	*Dendroica vitellina vitellina*	GC	Insects	Near Threatened
Western Spindalis (previously Stripe-headed Tanager)	*Spindalis zena salvini*	GC	Insects and small lizards	
Bullfinch	*Melopyrrha nigra taylori*	GC	Seeds fruit and insects	Scarce
Greater Antillean Grackle	*Quiscalus niger caymanensis*	GC	Insects and seeds	

Sources; Cayman Wildlife, Bradley et al

Although the Indian whistling duck (*Dendrocygna arborea*), and white winged dove (*Zenedia asiatica)* are mentioned in the Cayman NABP 2008 as an important species for urban areas they do not feed on insects and therefore are excluded as they will not be exposed to the *Aedes aegypti* mosquitoes.

c) **Terrestrial Amphibians and Reptiles**

A total of 24 indigenous and 15 introduced species of amphibian and reptile are currently known from the Cayman Islands (one species has both indigenous and introduced populations so the total for all herpetofauna is 38 species). Their fairly remote location means that the Cayman Islands

support 11 endemic species, over 45% of the total indigenous herpetofauna and the highest proportion for any UK Overseas Territory, including six lizards and five snakes (Edgar,2009). The threatened and endangered amphibians and reptiles have been reviewed in Edgar, 2009. The NBAP for Cayman lists the key species for urban areas as:

Common name	Latin name	Status (CITES, IUCN Red list)
Eastern Grand Cayman Blue throated Anole	*Anolis conspesus lewisi*	Declining in numbers due to habitat loss
Grand Cayman Racer	*Alsophis cantherigerus caymanus*	Unknown status
Grand Cayman Water Snake	*Tretanorhinus variabilis lewisi*	Unknown status
Grand Cayman Ground Boa	*Tropidophis caymanensis caymanensis*	Unknown status
Grand Cayman Blind snake	*Typhlops caymanensis*	Unknown status

d) Other species

It is likely that other invertebrates such as ants, termites etc might predate on mosquito eggs laid in oviposition sites such as the ovitraps containers etc. Dragonflies are also known to eat mosquito larvae. Spider species could also be potential predators.

3. Statement on the overall risk analysis

Risk analysis has been conducted on the hypothetical release of genetically modified *Aedes aegypti* mosquitoes expressing a self-limiting trait and a marker gene, as present in strain OX513A. The independent risk assessment was conducted by over 70 Malaysian scientists as part of a UNDP/University of Malaya sponsored workshop on the Risk Assessment of Transgenic Insects in Nov 2008. The proceedings of the workshop have been written up and are available (Beech et al, 2009).

The conclusions of the risk assessment were that a potential release of OX513A male mosquitoes would have a negligible risk to human health or the environment, although certain risks were identified as low risk from the trial:

a) Ecological niche replacement and potential increase of disease transmission by the secondary vector of dengue, *Aedes albopictus*. This is not of concern for a limited duration trial as ecological niche replacement is only likely to happen over an extended time period and could be monitored.
b) Alteration of food chains or webs by the removal of *Aedes aegypti* from the environment. Again this is not a concern for a limited duration trial, where the objective is not species suppression or eradication. Additionally *Aedes aegypti* is an introduced species, there are no species that are obligate on a single species of mosquito and there are many other mosquito species on which predators and prey can feed.

c) The potential for genetically modified *Aedes aegypti* to be less susceptible to insecticides used in control regimes and to transfer such reduced susceptibility to the wild population.
Insertion of transgenes, e.g. fluorescent marker genes, or modification of the expression of endogenous genes due to the insertion of such transgenes may have the potential to make a transgenic mosquito strain less susceptible to insecticides used in control regimes. However, the LMO strain for the proposed trial (OX513A) has been tested against the range of insecticides currently used was found to be equally susceptible as local strains. .

As the proposed release is short in duration, limited in scope and the *Aedes aegypti* strain cannot establish in the environment, due to the self-limiting genetic lethality i.e the progeny will die, there are not likely to be any pathogenic or/and ecologically disruptive effects.

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