ASSESSMENT OF GENETICALLY MODIFIED MOSQUITO EXHIBITING DOMINANT LETHALITY

Event: OX513A

Adapted from information available on the Biosafety Clearing House and the world wide web. Information on this case study may not be identical of those on official submissions or documents of the event OX513A. Information has been modified for training purposes.

SECTION I. INFORMATION OF THE LIVING MODIFIED ORGANISM

1. Genetically modified mosquito

1.1 overview

A transgenic strain of the *Aedes aegypti* mosquito, designated as OX513A(My1), was developed to exhibit dominant lethality in both males and females when reared in the absence of a transgene repressor as well as a dominant fluorescent marking to identify the transgenic insects. The LMO's name is: "Dominant lethal *Aedes aegypti* mosquito", and the transformation event is OX513A.

Application information: This application is being submitted for importation of eggs and eventual release of GM mosquitos into the environment. The goal is the suppression of the local *Aedes Aegypti* population. The process will involve the importation of eggs, that will be then kept in local facilities. Once mosquitos are ready, male OX513A populations will be release in several phases, quantities depending on local populations (monitoring).

1.2 Characteristics of the transformation process

Vector: pLA513 and phsp-pBac

Techniques used for the modification: Microinjection

Genetic elements:

-Actin 5c gene Promotor (P-Act5C): Promoter from *Drosophila melanogaster* - Common Fruit Fly.

-DsRed2 Fluorescent Protein (CS-DsRed2): Protein coding sequence, acts as a selectable marker. DsRed2 is a highly engineered monomeric form of the normally tetrameric red fluorescent protein variant of Discosoma sp. red **fluorescent protein** (drFP583; 1) with faster maturation and lower non-specific aggregation. The DsRed2 sequence contains a series of silent base-pair changes corresponding to human codon-usage preferences for high expression in mammalian cells.

-Dorsomycin gene 3'UTR (T-drs): Terminator. Drosomycin is the first antifungal protein characterized recently among the broad family of inducible peptides and proteins produced by insects to respond to bacterial or septic injuries. It is a small protein of 44 amino acid residues extracted from *Drosophila melanogaster* that exhibits a potent activity against filamentous fungi.

-tet Operator Sequences (BS-tetO-ECOLX): Binding site from *Escherichia coli.*

- HSP70 minimal promoter (P-HSP70) from *Drosophila melanogaster.*

-Tetracycline-controlled transactivator (CS-tTAV): Protein coding sequence. The name of the protein expressed is Tetracycline-controlled transactivator, it produces **conditional lethality**. Tetracycline-controlled transactivator (tTAV) is a synthetic protein coding sequence based on a fusion of sequences from *Escherichia coli* and *Herpes simplex* virus (VP16 transcriptional activator).

tTAV is under the control of its own binding site, tetO. In the absence of tetracycline, tTAV binds to tetO and drives expression of more tTAV, in a positive feedback loop. In the presence of tetracycline, tTAV binds tetracycline; this tetracycline-bound form does not bind tetO and so does not lead to expression of more tTAV. Consequently, this construct gives very high levels of expression of tTAV in the absence of tetracycline, but only low, basal expression in the presence of tetracycline.

tTAV encodes a dominant trait that, when introduced into certain insects, causes death of the insect unless the antibiotic tetracycline is supplied.

- fs(1)K10 3' UTR (T-fs(1)K10): terminator from *Drosophila melanogaster* .The expression of the fs(1)K1O gene is required in early oogenesis for the establishment of the dorsal -ventral polarity of the oocyte, and later in the embryo.

1.3 The Living modified organism

LMO characteristics: The LMO exhibits changes in reproduction. OX513A(My1) is a bisex RIDL strain, which means that both female and male insects die unless supplied with the supplement, which in the case of OX513A(My1) is the antibiotic tetracycline.
Released bisex RIDL insects and their progeny die within a few weeks so releases must be sustained to maintain the control.

Information on the Release of Insects carrying a Dominant Lethal (RIDL) technology

Release of Insects carrying a Dominant Lethal (RIDL) is a method using recombinant DNA technology to create genetically modified insects for biological control. The dominant lethal gene kills the insects but it can be repressed by an external additive, which allows the insects to be reared in manufacturing facilities. This external additive is commonly administered orally, and so can be an additive to the insect food. The insects can also be given genetic markers, such as fluorescence, that make monitoring the progress of eradication easier.

There are potentially several types of RIDL, but the more advanced forms have a female-specific dominant lethal gene. This avoids the need for a separate sex separation step, as the repressor can be withdrawn from the final stage of rearing, leaving only males.

These males are then released in large numbers into the affected region. The released males are not sterile, but any female offspring their mates produce will have the dominant lethal gene expressed, and so will die. The number of females in the wild population will therefore decline, causing the overall population to decline. Using RIDL means that the males will not have to be sterilized by radiation before release (as done with the "Sterile Insect Technique" (SIT) using radiation), making the males healthier when they need to compete with the wild males for mates.

Common use(s): Biological control

The process:

1) Genes (conditional lethality and fluorescent marker) are inserted into mosquito eggs
2) The lethality gene makes the mosquitos dependent on tetracycline.
3) After the first GM mosquitos grow into adults, they are breed in the lab.
4) Genetic modification is passed on to new generation producing a colony totally dependent on tetracycline.
5) The eggs from that colony are collected and placed in water where they become larvae and then pupils.
6) Males are separated from females. Male pupils are smaller (* only male mosquitos will be released! Only female mosquitos bites).
7) Feeding them tetracycline keeps the male mosquitos alive until they are released into the field.
8) Male mosquitos are released in the field where they are expected to mate with wild female mosquitos and pass the lethality gene. (male carries 2 copies of the lethality gene so it is always inhered).
9) Offspring will not survive in absence of tetracycline and will die.
10) The method for releasing the mosquitos into the wild is done using a car from where batches of mosquitos are released periodically on an specific area.

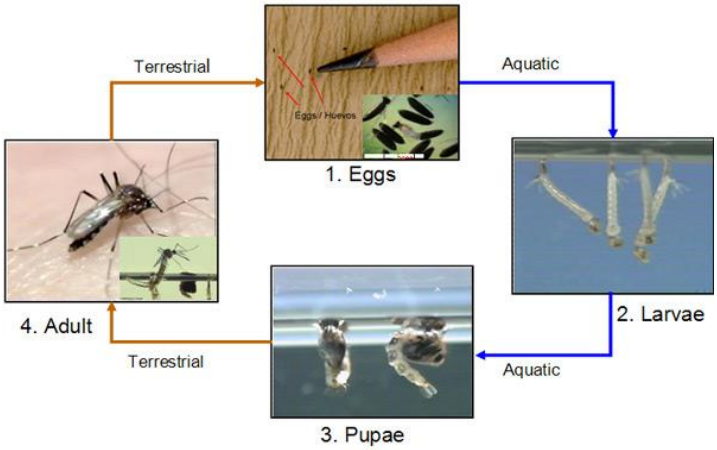
SECTION II. THE UNMODIFIED RECIPIENT ORGANISM

2.1 Taxonomic classification

Kingdom Animalia
Phylum Arthropoda
Class Insecta
Order Diptera
Family Culicidae
Genus *Aedes*
Species *aegypti*

2.2 The organism

Type of organism: insect
Domestication: wild
Centre of origin: *Aedes aegypti* originated in tropical Africa (North Africa) and achieved pan-tropical distribution in the 1930's.
Centre of genetic diversity: Tropical Africa (North Africa)
Habitat range: Tropical and sub-tropical region
Known pathogenicity and/or allergenicity: Vector for dengue, yellow fever, zika and chikungunya
Life cycle: *Aedes aegypti* and other mosquitoes have a complex life -cycle with dramatic changes in shape, function, and habitat. Female mosquitoes lay their eggs on the inner, wet walls of containers with water. Larvae hatch (picture 1, inset) when water inundates the eggs, as a result of rains or the addition of water by people. In the following days, the larvae (picture 2) will feed on microorganisms and particulate organic matter, shedding their skins three times to be able to grow from first to fourth instars. When the larva has acquired enough energy and size and is in the fourth instar, metamorphosis is triggered, changing the larva into a pupa (picture 3). Pupae do not feed; they just change in form until the body of the adult, flying mosquito is formed. Then, the newly formed adult emerges from the water after breaking the pupal skin (picture 4, inset). The entire life cycle lasts 8-10 days at room temperature, depending on the level of feeding. Thus, there is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults) in the *Ae. aegypti* life-cycle.



There is a very important adaptation of vectors that makes controlling their populations a difficult task. Their eggs can withstand desiccation for several months, which means that even if all larvae, pupae, and adults were eliminated at some point in time, repopulation will occur as soon as the eggs in the containers are flooded with water. Unfortunately, there is no effective way to control the eggs in containers.

SECTION III. THE RECEIVING ENVIRONMENT

3.1 General

Name of the country: Republic of Calgu
Area: 207,595 km^2(80,153 sq mi)
Population: 8,504,700 (2016 estimate)

3.2 Geography and Climate

Calgu is a country located in south America, and it has 2 geographic regions: coastal regions and mountains. Its climate is characterized for being tropical and isothermal as a result of its geographical location near the Equator. The striking variety in temperature and precipitation results principally from differences in elevation. Temperatures range from very hot at sea level to relatively cold at higher elevations but vary little with the season. In the coastal areas, temperature can vary from 24 and 38 °C. In the mountain areas temperatures go from 10 and 19 °C.

3.3 Biodiversity

More than 40,000 species are registered in Calgu of which 5,000 are endemic. The country occupies an important position worldwide in number of birds, and plants.

The country hosts 30 nationally designated protected areas. According to reports, half of Calgu's ecosystems are in a critical state of deterioration or in a state of danger. The organization said that environmental degradation is due to oil extraction, mineral and metal extraction and deforestation. Deteriorating ecosystems are threatening the existence of more than a third of Calgu's plants and 40 percent of its animals

3.4 Health issues related to vector-borne

Most illnesses are the result of Calgu's tropical-zone location. If traveling anywhere along the coast or jungle, you can bank on little tropical nuisances – infected bug bites, rashes or heat exhaustion. Other, more dangerous afflictions, including malaria, dengue, zika, chikungunya and yellow fever, can strike.

Dengue fever and the newest mosquito-borne threat, chikungunya, which arrived in 2010 on Calgu's shores, and zika, which hit the country hard in 2015, are a risk in lowland population centers although the epidemic has passed and not many cases are being reported at present. Other problems can occur in the mountains, including soroche (altitude sickness).