# For Public Comment

# Preliminary
# FINDING OF NO SIGNIFICANT IMPACT

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**Prepared by the**
**Center for Veterinary Medicine**
**United States Food and Drug Administration**
**Department of Health and Human Services**

**Preliminary Finding of No Significant Impact (FONSI)
In Support of an Investigational Field Trial of
OX513A *Aedes aegypti* Mosquitoes**

The US Food and Drug Administration (FDA)'s Center for Veterinary Medicine (CVM) received a draft environmental assessment (EA) from Oxitec Ltd. (the sponsor) for its proposed investigational field trial of genetically engineered (GE) male *Aedes aegypti* mosquitoes, strain OX513A, under an investigational new animal drug (INAD) exemption (21 CFR 511.1(b)). The proposed investigational field trial would be carried out in Key Haven, Monroe County, Florida under Oxitec's supervision in conjunction with the Florida Keys Mosquito Control District (FKMCD). *Ae. aegypti* is a known vector for human diseases including dengue fever and chikungunya.

The sponsor's draft EA is being posted for public comment.[1] This preliminary FONSI is based on FDA's evaluation of the data and information included in the sponsor's draft EA, other data submitted by the sponsor, observations made by FDA inspectors accompanied by a subject matter expert from CDC during an inspection of the Hatching and Rearing Unit (HRU), and a visit to the proposed field study site. Evaluation of the sponsor's draft EA and preparation of these preliminary findings were carried out by members of CVM's Animal Biotechnology Interdisciplinary Group (ABIG), experts from the Centers for Disease Control and Prevention (CDC), and the Environmental Protection Agency (EPA) who together serve as the FDA inter-agency review team ("the review team") consistent with the Coordinated Framework for the Regulation of Biotechnology.[2, 3] All findings, conclusions, or determinations described in this document are preliminary and may change based on further review.

OX513A mosquitoes have been genetically engineered to encode a conditional or repressible lethality trait, which is a function of the overexpression of the tetracycline-repressible transactivator (tTAV) protein, and a red fluorescent marker protein. When tetracycline is not present (i.e., upon release of OX513A mosquitoes to the environment as in the proposed investigational field trial), tTAV causes lethality in the progeny of matings between OX513A males and wild-type females. The fluorescent marker can be used to identify the GE mosquitoes as larvae and pupae in the laboratory. As described in the draft EA, OX513A eggs would be produced by Oxitec in Oxford, UK and shipped to Marathon, FL for rearing in the HRU at a FKMCD facility. Male OX513A mosquitoes produced in the HRU would be used for the proposed investigational field trial. The goals of the proposed investigational trial are to evaluate the breeding of the OX513A mosquitoes with local wild-type *A. aegypti* females, to assess the survival of the resultant progeny, and to estimate the suppression of the overall *Ae. aegypti* population at the trial site relative to an untreated comparator area. At the conclusion of the investigational field trial, the OX513A mosquitoes would die off at the end of their natural lifetimes in the environment (approximately two days) and wild-type *Ae. aegypti* levels are expected to recover to pre-trial numbers.

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[1] http://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/UCM487377.pdf

[2] https://www.whitehouse.gov/sites/default/files/microsites/ostp/57_fed_reg_6753__1992.pdf

[3] The preliminary conclusions in this preliminary FONSI have been made by the FDA with the review team's concurrence.

FDA's evaluation of data and information in the draft EA was based on Oxitec's characterization of potential hazards, potential exposure pathways, and the likelihood of risk associated with investigational use of OX513A mosquitoes. In its review of the draft EA the review team evaluated potential impacts associated with the proposed investigational trial such as impacts on human and animal health, impacts on ecosystem, and impacts of the introduced trait on existing and introduced populations of *Ae. aegypti*. FDA analyzed these impacts in a risk context: characterization of hazard, characterization of exposure pathway and receptors (i.e., individuals or populations experiencing the exposure), estimation of risk, and characterization of level of uncertainty regarding risk estimate. Because risk is a function of hazard and exposure, if exposures are negligible, risk will also be negligible.[4]

These hazards and risks are described below, and FDA's preliminary findings drawn from that body of work form the basis of this preliminary FONSI.

*Potential impacts on human or animal health*

The potential impacts on human or animal health include the risk of toxic or allergic reactions caused by exposure to the tTAV or DsRed2 proteins, or the #OX513 rDNA construct in OX513A mosquitoes, or potential increase in transmission of dengue or other viral diseases spread by *Ae. aegypti*.

FDA found that the probability that the release of OX513A male mosquitoes would result in toxic or allergenic effects in humans or other animals is negligible based on the sponsor's draft EA. Almost all of the OX513A mosquitoes released for the investigational field trial will be male, and male mosquitoes do not bite humans or other animals. They are therefore not expected to have any direct impacts on human or animal health.

Based on the data and information found in the sponsor's draft EA, FDA found that it is highly unlikely that humans or animals bitten by an OX513A female mosquito would be exposed to tTAV or DsRed2 proteins. This conclusion is based on studies demonstrating that concentrations of these proteins in the saliva of OX513A females are below the limit of detection in assays performed to detect the level of these proteins in OX513A female mosquito saliva. Further, the bioinformatics analysis done by the sponsor, and the opinions of allergenicity experts submitted by the sponsor, indicated that there was no convincing evidence to suggest that tTAV and DsRed2 proteins represent risks of allergy or toxicity. Therefore, based on the data and information presented, FDA concluded that that the immunological response in humans and animals to OX513A female mosquito bites is not expected to be different from the immunological response to bites by wild type *Ae. aegypti* mosquitoes.

It is also highly unlikely that the #OX513 rDNA construct itself would have any adverse impacts on humans or any other animals. The FDA inter-agency review team established that the #OX513 construct is stably integrated in the OX513A mosquito genome and completely refractory to remobilization, even when deliberately re-exposed to *piggyBac* transposase. Therefore, it is highly unlikely that the #OX513 rDNA construct could be transferred to humans or other animals and pose a higher risk to them than would be expected from any mosquito bite.

FDA found that it is highly unlikely that release of OX513A male mosquitoes would contribute to the increase in transmission of dengue or other diseases transmitted by mosquitoes. Male mosquitoes do

[4] National Research Council. 2002. *Animal Biotechnology: Science Based Concerns.* NAS Press.

not bite humans or other animals and therefore do not transmit diseases. Further, their environmental lifetimes are short (~2 days), limiting their ability to interact with humans.

Blood feeding of OX513A females as a requirement for egg laying is performed in Oxitec's UK facilities. Blood used for this process is collected using a sterile apparatus and processed aseptically from a closed herd of healthy animals permanently housed in UK, under regular veterinarian supervision, that are screened for virus, bacteria, and other pathogens to minimize the potential for contamination of the final defibrinated blood product. The host range of *Aedes aegypti* does not extend to the UK and, therefore, the risk of arbovirus such as dengue and chikungunya transmission to these animals is negligible. As a result, the blood collected from the horses would be free of such arboviruses. The number of OX513A female mosquitoes that potentially may be co-released or present at the proposed trial site as a result of incomplete penetrance of the lethality trait (i.e., do not express the lethality trait) is expected to be extremely low. In addition, it would be highly unlikely that the OX513A mosquitoes would be exposed to human disease viruses under containment at the HRU, and therefore the risk of their carrying the viruses that cause dengue or other diseases is negligible.

It is unlikely that suppression of *Ae. aegypti* at the proposed investigational trial site would lead to an increase in population of other mosquito species that may transmit other diseases due to the short duration of the proposed investigational trial. *Ae. aegypti* occupy a fairly uncontested ecological niche; their absence would not likely provide an opportunity for other mosquitoes to move in and expand to fill it. The population of *Ae. aegypti* at the proposed site is expected to return to its original levels upon completion of the proposed investigational trial due to migration of wild type *Ae. aegypti* from areas that did not receive OX513A male mosquitoes.

Based on the data and information submitted in the draft EA, other submissions from the sponsor, and scientific literature, FDA found that the probability of adverse impacts on human or other animal health is negligible or low.

*Potential impacts on the ecosystem*

FDA found that the probability that the release or rearing of OX513A *Ae. aegypti* would have adverse impacts on the ecosystem is largely negligible; a finding of low risk was found for the potential for tetracycline in the environment to kill bacteria involved in environmental processes.

*Ae. aegypti* are a uniquely peri-domestic species adapted to living in areas populated by humans. Immature stages of *Ae. aegypti* are usually found in fresh water collected in puddles or man-made containers such as gutters, containers, and discarded tires, etc. Because *Ae. aegypti* breed in peri-domestic environments, they are subject to opportunistic predators that prey on their larvae and adults, if and when they encounter them. FDA did not identify any specific parasitoid species associated with *Ae. aegypti,* with the exception of generalist parasitoids infecting a number of mosquito species. In addition, no decomposers specific to *Ae. aegypti* were identified nor is *Ae. aegypti* a specific decomposer of detritus. There are no reports indicating that *Ae. aegypti* mosquitoes are a pollinator for any plant species. Further, upon completion of the proposed investigational trial, the population of *Ae. aegypti* is expected to be restored to its pre-field trial population level.

Section 7(a) of the Endangered Species Act (ESA) requires federal agencies to "insure that any action authorized, funded, or carried out by the agency" (the agency action) "is not likely to jeopardize" the continued existence (or result in the destruction or adverse modification of a designated critical habitat)

of any species of fish, wildlife, or plants that have been determined to be threatened or endangered under Section 4 of the ESA (i.e., officially listed). One of the first steps in this process is a determination by the action agency (FDA in this case), as to whether the proposed action "may affect" listed species or critical habitat (FWS/NMFS, 1998). There is one endangered species, the Stock Island Tree Snail, whose habitat is in the vicinity of the proposed investigational field trial area. The draft EA determined that the proposed investigational use of OX513A mosquitoes would not adversely affect Stock Island Tree Snails because the Stock Island Tree Snail's habitat (hammock and beach berm) does not overlap with the domestic or peri-domestic environment of *Ae. aegypti*. Additionally, the proposed investigational trial does not propose to remove or modify the snail's habitat (hammock and beach berm). Consequently, the FDA made a "no effect" determination under the ESA, 16 U.S.C. § 1531 et seq. The proposed investigational trial, as described in the draft EA, would not jeopardize the continued existence of the endangered Stock Island Tree Snail or result in the destruction or adverse modification of their critical habitat. Additionally, the proposed investigational trial would not jeopardize the continued existence of any other endangered species in wildlife refuges located in Monroe County or result in the destruction or adverse modification of other endangered species' critical habitat due to their being located at least 18 miles from the proposed investigational trial site, which considerably exceeds the flight range of *Ae. aegypti* mosquitoes.

Based on information provided in the draft EA and the inspection of the HRU proposed for the rearing of OX513A mosquitoes, the probability of adverse impacts associated with rearing of OX513A mosquitoes was found to be extremely unlikely. OX513A mosquito rearing would be carried out at the HRU, a specialized facility that conforms to the Arthropod Containment Level 2 (ACL2) guidelines[5] developed by the American Committee on Medical Entomology, and has multiple redundant levels of physical containment as confirmed by the inspection. Disposal of adult and other life stages of mosquitoes at the HRU would be carried out according to the ACL2 guidelines, thus posing no significant risk to the personnel working at the HRU and the environment.

*Potential impacts associated with the failure of the introduced trait in OX513A mosquitoes*

FDA established that the probability of OX513A mosquitoes and their progeny persisting and establishing at the proposed trial site or spreading beyond its boundaries is extremely unlikely. The OX513A line of *Ae. aegypti* mosquitoes carries a repressible dominant lethality trait that does not allow their survival or the development of their progeny past the late larval or early pupal stage unless reared in the presence of tetracycline. The proposed investigational trial site does not have any sources of tetracycline that are sufficiently high to allow survival of OX513A progeny in the environment. Persistence or establishment of OX513A mosquitoes is also highly unlikely due to the introduced lethality trait. The location of the proposed investigational trial site is also expected to limit dispersion of OX513A mosquitoes due to its relative isolation and existing natural geophysical barriers. Further, given that the proposed investigational trial would be carried out concurrently with the existing FKMCD integrated pest management mosquito control measures currently in place, it is extremely unlikely that OX513A mosquitoes would disperse beyond the trial area.

It is highly unlikely that OX513A mosquitoes and their progeny would develop resistance to insecticides commonly used in mosquito control, as the OX513A mosquitoes' susceptibility to insecticides commonly used in mosquito control is similar to that of wild type *Ae. aegypti* mosquitoes.

[5] http://www.astmh.org/AM/Template.cfm?Section=ACME&Template=/CM/ContentDisplay.cfm&ContentID=1450

As required by the National Environmental Policy Act (NEPA) and its implementing regulations, alternatives to the proposed action were evaluated. The alternative that the review team evaluated was the no action alternative (i.e., Oxitec would not carry out the investigational field trial in Key Haven, Florida). There are two likely scenarios under the no action alternative: (1) the investigational field trial would not be conducted in Key Haven, FL and Oxitec would continue development and commercialization of the OX513A mosquitoes at locations outside the United States; or (2) Oxitec would select another location in the United States in which it would conduct the investigational field trials. There would be no major federal action arising from the first scenario, and therefore no potential environmental impacts for the agency assess under NEPA. Should Oxitec not conduct an investigational field trial anywhere else in the United States or its territories, the results of the alternative action would be the current status quo. Although there could be environmental impacts if Oxitec determined that it wanted to conduct an investigational field trial elsewhere in the United States, it would have to prepare an environmental assessment of that trial in the alternative selected location and that environmental assessment would evaluate the potential impacts.

*NEPA Decision and Findings*

As previously noted, the review team that includes experts from CVM, EPA, and CDC has carefully considered the potential environmental impact of the proposed investigational trial and the no action alternative. The consequences of escape, survival, and establishment of OX513A in the environment have been extensively studied: data and information from those studies indicate that there are unlikely to be any adverse effects on non-target species, including humans. Risk of establishment or spread has been determined to be negligible. The investigational trial is short in duration and any unanticipated adverse effects are unlikely to be widespread or persistent in the environment. Most importantly, the status of the environment is restored when releases are stopped (i.e., the released mosquitoes all die, and the environment reverts to the pre-trial status). FDA has therefore made the preliminary finding that the proposed field trial would not individually or cumulatively have a significant effect on the quality of the human environment in the United States, and is issuing this preliminary FONSI.