FINDING OF NO SIGNIFICANT IMPACT

In support of a proposed field trial of genetically engineered (GE) male *Aedes aegypti* mosquitoes of the line OX513A in Key Haven, Monroe County, Florida under an investigational new animal drug exemption

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Center for Veterinary Medicine
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GE male *Aedes aegypti* mosquitoes of the line OX513A
In Key Haven, Monroe County, Florida
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Oxitec, Ltd. (Oxitec, the sponsor) has provided data and information to the U.S. Food and Drug Administration (FDA)'s Center for Veterinary Medicine (CVM, we) for its proposed field trial of genetically engineered (GE) male *Aedes aegypti* mosquitoes of the line OX513A, under an investigational new animal drug (INAD) exemption (21 CFR 511.1(b)). *Ae. aegypti* is a vector for human diseases including those associated with Zika, dengue, and chikungunya viruses. OX513A have been genetically engineered to express a gene that encodes a conditional or repressible lethality trait (also known as self-limiting) and a red fluorescent marker protein to aid in their identification. 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).

Consistent with the mandates in the National Environmental Policy Act of 1969 (NEPA), 42 U.S.C. § 4321 et seq. and FDA's environmental impact considerations regulations (21 CFR part 25), FDA has thoroughly evaluated the potential environmental impacts associated with the proposed field trial. CVM determined hazards and pathways to harm and evaluated risks with expert opinion from members of the FDA inter-agency review team ("the review team") from the Centers for Disease Control and Prevention (CDC), and the Environmental Protection Agency (EPA) consistent with the Coordinated Framework for the Regulation of Biotechnology.[1] FDA posted Oxitec's draft Environmental Assessment (EA) and FDA's preliminary FONSI for public comment on March 11, 2016 (81 FR 13371), took relevant comments under consideration, and prepared the attached final EA dated August 5, 2016. This FONSI is based on analyses and findings presented in the final EA, including a consideration and evaluation of alternatives. In this case, the alternative evaluated was the no action alternative (i.e., Oxitec would not carry out the investigational field trial in Key Haven, Florida) and two likely scenarios under that alternative: (1) the investigational field trial would not be conducted in Key Haven 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 for the conduct of its investigational field trials. See EA, Section 8.1.

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 (DsRed2). When tetracycline is not present (i.e., upon release of OX513A mosquitoes into the environment as in the proposed field trial), tTAV causes lethality in mosquitoes carrying at least one copy of the #OX513 recombinant DNA (rDNA) construct including 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 under laboratory conditions. OX513A eggs would be produced by Oxitec in Oxford, UK and shipped to Marathon, Florida for rearing in a Hatching and Rearing Unit (HRU) at a FKMCD facility. Male OX513A mosquitoes produced in the HRU would be used for the proposed field trial. The goals of the proposed trial are to evaluate the breeding of OX513A male mosquitoes with local wild-type *A. aegypti* females, to assess the survival of the resultant progeny, and

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

to estimate the suppression of the overall *Ae. aegypti* population at the trial site (treatment area) relative to an untreated comparator area. At the conclusion of the proposed 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.

FDA's analysis in the EA is based on characterization of potential hazards, potential exposure pathways, and the likelihood of risk associated with investigational use of OX513A mosquitoes. In the EA, FDA evaluated potential impacts associated with the proposed field trial such as impacts on human and animal health and impacts on the environment. 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 the level of uncertainty regarding the risk estimate. Because risk is a function of hazard and exposure, if exposures are negligible, risk will also be negligible.[2]

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

Potential impacts on human or non-target animal health

The potential impacts on human or non-target animal health include potential toxic effects in humans or non-target animals or allergenic effects in humans, transfer of the rDNA construct to humans or non-target animals, increase in transmission of dengue or other diseases transmitted by mosquitoes, increase in population of other mosquitoes that is opportunistic or via niche expansion that may contribute to the increase of disease, development of antimicrobial resistance, inadvertent release of OX513A females at the trial site, and a failure of the introduced traits in OX513A mosquitoes.

FDA found that the probability that the release of OX513A male mosquitoes would result in toxic effects in humans or non-target animals or allergenic effects in humans is extremely low and the risk is negligible. Almost all of the OX513A mosquitoes released as part of the proposed field trial will be male, and male mosquitoes do not bite humans or other animals. The trial protocol uses a sex sorting method based on the size difference between male and female pupae with quality control processes that ensure accuracy of sorting does not exceed a maximum of 0.2%. Thus, the overall probability of an OX513A female mosquito being released during the investigational trial is very low (0.2% at most) and the probability of this released female locating a human host and taking a blood meal is also low based on the estimated total human population in the trial area. Further, results from Western immunoblot assays performed by Oxitec indicate the Limit of Detection (LOD) to be 0.8 ng and 2.5-5 ng for tTAV and DsRed2 respectively when the amount of salivary protein used per sample was four times what is injected into a human host in a single bite (i.e., at approximately 0.2 (tTAV) and 0.625-1.25 ng (DsRed2) for the amount of protein injected in a single bite). Because both tTAV and DsRed2 proteins were undetectable by this assay the data supports the hypothesis that, if they are expressed and secreted in saliva at all, these proteins are likely expressed below or close to the 1 ng range per *Aedes* female bite, which is much lower than the level at which known human allergens in mosquito saliva are expressed. The likelihood that tTAV and DsRed2 would cause an allergic response in a human host that is bitten by an OX513A female is, therefore, extremely low. Oxitec also performed a bioinformatics analysis as per

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

Codex Alimentarius guidelines (2003; 2009) to determine potential IgE binding epitopes as well as the potential for cross-reaction with other known human allergens. This analysis did not return results that were a significant match to known allergens or allergenic epitopes. Taken together these data suggest that there are unlikely to be epitopes that are known to cause allergenic reactions in humans.

The likelihood that the transfer of the rDNA construct to humans or non-target animals could result in an adverse effect is extremely low and the risk is negligible. FDA determined that it is highly unlikely that the #OX513 rDNA construct could be transferred to humans or animals via biting because it is stably integrated in the mosquito genome and is not capable of re-mobilization even when treated with appropriate transposases and there is no known pathway for the naked, full length #OX513 rDNA to be present in mosquito saliva. Additionally, mosquitoes have been feeding on humans and other animals for millennia but there is no evidence of DNA transfer between mosquitoes and humans or animals. Further, we determined that #OX513 rDNA construct transfer to microorganisms (e.g., bacteria in the intestine or surface of OX513A mosquitoes, humans, or other animals; bacteria present in soil and involved in decomposition of organic matter) is highly unlikely due to a number of physical, biochemical, and genetic barriers that restrict horizontal gene transfer. Despite the fact that prokaryotes are exposed to an abundance of genetic material from eukaryotic organisms, the presence of eukaryotic genes in the genomes of prokaryotes is extremely limited and suggests the existence of functional and selective barriers that limit the acquisition of eukaryotic genes by bacteria. Therefore, FDA concludes that the likelihood of adverse effects associated with a potential transfer of the rDNA construct to humans or other non-target animals is extremely low and the risk is negligible.

The likelihood that the release of OX513A mosquitoes would result in an increase in transmission of dengue or other diseases transmitted by mosquitoes is extremely low and the risk is negligible. OX513A male mosquitoes do not bite and, consequently, do not transmit diseases. A small number of females may be co-released with OX513A male mosquitoes or be present at the site of the proposed release as a result of incomplete penetrance of the introduced lethality trait. However, there is no evidence to suggest that OX513A females are fitter or more competent vectors than wild-type *Aedes aegypti*. In fact, evidence suggests OX513A females have decreased vector competence because any OX513A females are expected to die in 2-3 days time, as the lack of tetracycline in the environment will turn on the lethality trait resulting in a lifespan too short to vector viral disease. The lifespan of OX513A females is shorter than the external incubation period, (EIP) for arboviruses such as dengue and Zika thereby disabling virus transmission to a human host at a subsequent blood feeding. Further, disease transmission by OX513A females requires that they can locate a human host that is infected with a sufficient titer of virus and blood feed adequately, that the female's lifespan is sufficiently long to allow virus multiplication and secretion into saliva, and that the female lives long enough to blood feed again after the EIP is complete, thereby transmitting the virus to a human host. EIP for dengue is estimated at 10-14 days. All of these factors combined suggest that, if anything, OX513A females would have a lower overall vectorial capacity as compared to wild-type *Ae. aegypti*. In addition, OX513A mosquitoes would be produced under disease-free conditions that further limit the possibility of transmitting any diseases. Therefore, FDA concludes that the likelihood of adverse effects associated with an increase in transmission of dengue or other diseases transmitted by OX513A mosquitoes is extremely low and the risk is negligible.

The likelihood that the release of OX513A mosquitoes would lead to an increase in the population of other mosquito species that is opportunistic or via niche expansion that might contribute to an increase in disease transmission at the proposed trial site is extremely low and the risk is negligible. A suppression field trial using OX513A in Panama resulted in an 82% suppression of *Ae. aegypti* over an

84-day period without an increase in *Ae. albopictus* at the same site.[3] As discussed in the EA, this suggests that a short term field trial as proposed for Key Haven, Florida should not have an effect on local *Ae. albopictus* populations via niche expansion. Additionally, the wild-type *Ae. aegypti* population would be expected to recover to pre-trial numbers after the cessation of OX513A mosquito releases. Therefore, the likelihood of adverse effects associated with increase in population of other mosquitoes that may contribute to the increase of diseases at the proposed trial site is extremely low.

The likelihood that the production and release of OX513A mosquitoes would lead to development of antimicrobial resistant prokaryotes is extremely low and the risk is negligible. This is in part because resistant bacteria, even if present in the larval or pupal stages, would be highly unlikely to be present in adult OX513A mosquitoes because their gut bacteria are lost during mosquito metamorphosis from larvae to adults. The possibility of superficial bacteria present on the body surface of eclosed adults acquiring antibiotic resistance genes due to rearing conditions in the HRU is very low as there is no causal pathway for this to occur. Antimicrobial resistance arising in bacteria in the rearing water and the subsequent transfer of this trait to other bacteria that could cause food or water-borne diseases would also be highly unlikely due to the short duration of the mosquito life cycle as well as the trial in general. Waste water from the Hatching and Rearing Unit (HRU) is treated at a local waste water treatment facility in accordance with existing local and state laws, further precluding exposure of humans and non target animals to any potential bacteria, mosquito larvae, and substances used during rearing. Also process controls that would be implemented at the HRU (e.g., use of personal protective equipment) would eliminate the potential for transfer of antibiotic resistant bacteria to personnel involved in the production of OX513A mosquitoes. Therefore, FDA concludes that the likelihood of the adverse effects associated with development of anti-microbial resistance is extremely low and the risk is negligible.

The likelihood of adverse events due to the inadvertent release of OX513A females is extremely low and the risk is negligible due to standard operating procedures (SOPs) and quality control procedures that Oxitec would implement. FDA verified physical and procedural containment implemented at the HRU during an inspection. In the highly unlikely event that a person were bitten by an OX513A female inadvertently released at the trial site or by the female OX513A progeny that survived, the immunological response to these bites in humans and animals would not be expected to be any different from the immunological response to bites by wild-type *Ae. aegypti* mosquitoes as discussed above. In fact, we anticipate that it would be less dangerous in several respects: (1) released mosquitoes would be maintained in conditions and using husbandry procedures that prevent infection with virus, and (2) dengue virus has a longer EIP than the lifespan of short-lived OX513A females. Therefore, FDA concludes that the likelihood of the adverse effects associated with the release of OX513A females at the trial site is expected to be extremely low and the risk is negligible.

The likelihood that that the failure of the introduced traits in OX513A male mosquitoes would lead to any adverse effects is extremely low and the risk is negligible. The stability of the #OX513 rDNA construct was confirmed over multiple generations of OX513A mosquitoes. In the highly unlikely event that the introduced lethality trait is compromised, resulting in a loss of function of the tTAV lethality trait, these mosquitoes would be functionally no different and no fitter than wild-type *Ae. aegypti*.

[3] Gorman K, Young J, Pineda L, Marquez R, Sosa N, Bernal D, Torres R, Soto Y, Lacroix R, Naish N et al. 2016. Short-term suppression of *Aedes aegypti* using genetic control does not facilitate *Aedes albopictus*. *Pest Manag Sci* **72**: 618-628.

Oxitec would monitor the performance of OX513A mosquitoes during the proposed trial and would be able to detect the failure of the traits and respond appropriately including stopping the trial if necessary. Therefore, FDA concludes that the likelihood of the adverse effects associated with the failure of the introduced traits is expected to be extremely low and the risk is negligible.

Potential impacts on the environment

Potential impacts on the environment identified in the final EA include interbreeding with related mosquito species, effects of tetracycline on the environment, effects on flora, effects on predators, effects on decomposers, effects on endangered and threatened species, development of resistance to insecticides, and establishment of OX513A mosquitoes at the proposed trial site.

It is highly unlikely that OX513A males would interbreed with other, related mosquito species present at the proposed trial site as mating in mosquitoes is very species specific. Reproduction in *Ae. aegypti* is sexual and occurs in flight within aerial swarms. Mating behavior such as wing beat frequency and pitch of resonating antennae enable species identification and ensure species-specific mating. *Ae. aegypti* matings with closely related mosquito species do not produce viable offspring. Further, in the highly unlikely event that OX513A male mosquitoes do mate with other closely related mosquito species, it is highly unlikely that the rDNA construct would spread in the population of these mosquitoes due to the lethality phenotype conferred by this rDNA construct. Therefore, FDA concludes that the likelihood of adverse effects due to OX513A mosquitoes breeding with other mosquito species as well as the survival of any potential progeny produced is extremely low and the risk is negligible.

It is highly unlikely that the use of tetracycline in the production of OX513A mosquitoes would have any adverse effects on the environment. The levels of tetracycline in HRU waste water would be low (grams/week). These low levels are expected to be rapidly broken down in the environment as tetracycline is sensitive to light and rapidly degrades (with the bulk of degradation taking place on day 1) and has a short half-life in the environment. Therefore, FDA concludes that the likelihood of adverse effects associated with the use tetracycline for production of OX513A mosquitoes is extremely low and the risk is negligible.

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 trial, the population of *Ae. aegypti* is expected to be restored to its pre-field trial population level. FDA therefore concludes that the likelihood of adverse effects on the population of predators, parasitoids, decomposers, and flora is expected to be extremely low and the risk is estimated to be negligible.

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). There is one endangered species, the Stock Island Tree Snail, whose habitat is in the vicinity of the proposed field trial area. FDA 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 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 trial would not jeopardize the continued existence of the endangered Stock Island Tree Snail or result in the destruction or adverse modification of its critical habitat. Additionally, the proposed 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 trial site, which considerably exceeds the flight range of *Ae. aegypti* mosquitoes. Therefore, FDA concludes that the likelihood of adverse effects on threatened and endangered species is expected to be extremely low and the risk is estimated to be negligible.

It is highly unlikely that released OX513A mosquitoes would introduce insecticide resistance traits into the local *Ae. aegypti* mosquito population. Laboratory studies have shown that OX513A mosquitoes are susceptible to insecticides used for mosquito control. Therefore, FDA concludes that the likelihood of adverse effects associated with introduction of insecticide resistance into the local population of *Ae. aegypti* is expected to be extremely low and the risk is estimated to be negligible.

It is highly unlikely that OX513A mosquitoes would be able to establish at the proposed trial site. More than 95% of OX513A progeny die before reaching viable adulthood if reared without tetracycline. Our evaluation did not identify any sources at the proposed trial site that potentially could have levels of tetracycline sufficient to allow survival of OX513A progeny in the environment. Further, although the introduced lethality trait does not appear to have a significant effect on the mating competitiveness of OX513A males, it does appear to have a significant impact on longevity. Dispersal of OX513A mosquitoes appears to be adversely affected as measured by mean distance traveled, but not by maximum distance traveled, indicating that, in general, the population of OX513A mosquitoes is not expected to exhibit geographical dispersion significantly different from wild-type *Ae. aegypti*. The location of the proposed field trial site would also limit dispersion because of its relative isolation and existing natural geophysical barriers. Moreover, given that this trial would be carried out concurrently with the existing FKMCD integrated vector control program currently in place, it is unlikely that OX513A mosquitoes would disperse beyond the trial site. Therefore, FDA concludes that the likelihood of adverse effects associated with establishment of OX513A at the proposed trial site is extremely low and the risk is estimated to be negligible.

As defined by regulation, cumulative impacts are "the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal or non-Federal) or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time." 40 CFR 1508.7. As part of the environmental assessment, we have evaluated the environmental impacts of complete population suppression of Ae. *aegypti* at the proposed trial site, including effects on predatory animals, potential for niche expansion and risk of disease transmission. See Sections 13.5.2 and 14.2. Moreover, there are not expected to be any "incremental impacts" seen over time as a result of the proposed trial given that the local population of Ae. *aegypti* should revert to pre-trial levels once the experiment has stopped. Therefore, we have determined there would be no significant environmental impacts caused by the use of OX513A coupled

with the continued use of insecticides and other vector control methods employed at the proposed trial site and in the surrounding area that have not already been evaluated. Consideration of any future field trials at this time would be purely speculative. Thus, there are no cumulative impacts from this proposed action that would result in a significant environmental impact for which an EIS would be needed.

NEPA Decision and Findings

FDA has carefully considered the potential environmental impact of the proposed trial and the no action alternative, as described and evaluated in the EA. The consequences of escape, survival, and establishment of OX513A in the environment have been extensively studied: data and information from those studies indicate that the proposed investigational use of OX513A *Ae. aegypti* mosquitoes is not expected to cause any significant adverse impacts on the environment or human and non-target animal health beyond those caused by wild-type mosquitoes. FDA has, therefore, made the finding that the proposed field trial would not individually or cumulatively have a significant effect on the quality of the human environment. Based on that finding, FDA is issuing this FONSI and will not prepare an environmental impact statement.