



Keith A. Matthews

**VIA EMAIL
DOES NOT CONTAIN CBI**

May 20, 2019

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Re: Description of OX5034 *Aedes aegypti* Mosquito, including Active and Inert Ingredients

Dear Dr Bohnenblust:

On behalf of Oxitec Ltd. (Oxitec), we provide herein a description of the OX5034 *Aedes aegypti* mosquito, including a detailed description of the active and inert ingredients that give the OX5034 *Aedes aegypti* mosquito its uniquely safe and efficacious pesticidal properties. OX5034 *Aedes aegypti* is a homozygous diploid line of *Aedes aegypti* containing a single integrated copy of the #OX5034 rDNA construct that confers conditional lethality on female progeny of OX5034 *Aedes aegypti* mosquitoes.

The active ingredient of the OX5034 *Aedes aegypti* mosquito ("OX5034" or "OX5034 *Aedes aegypti*") is a tetracycline-repressible transactivator protein variant (tTAV-OX5034) and the genetic material necessary to produce the protein *in vivo* in female offspring of OX5034 *Aedes aegypti* matings. Female progeny inheriting the OX5034 rDNA construct express the tTAV-OX5034 protein as larvae and, in the absence of tetracycline or its analogues, die in L2/L3 larval instar stages, while males survive to fully functional adulthood. This means that released OX5034 *Aedes aegypti*, reared in the absence of tetracycline, will be males that cannot bite humans or other animals, and do not transmit disease.

The inert ingredient in OX5034 *Aedes aegypti* is a fluorescent marker, DsRed2-OX5034, which aids in the detection of *Aedes aegypti* carrying the #OX5034 rDNA construct. The DsRed2 protein belongs to a family of red fluorescent proteins, which are members of a group of fluorescent proteins identified in several *Anthozoa* species. DsRed2 is a synthetically modified variant of the original red fluorescent protein isolated from a coral-like anemone, *Discosoma* spp.

Similarities and differences between OX5034 and OX513A *Aedes aegypti* mosquitoes

Oxitec's 1st generation self-limiting mosquito technology (OX513A), successfully deployed in

multiple locations including in Brazil, the Cayman Islands and Panama, has been succeeded by the new 2nd generation self-limiting mosquito, OX5034. The OX5034 mosquito carries many of the key features of OX513A that made it a safe, effective control method for reducing *Aedes aegypti* mosquito populations. These include effective mosquito control, non-toxic and non-allergenic active and inert ingredients, a lack of direct effects on non-targeted species, and no long-term effects or chemical residues in the environment. OX5034 also has several additional features, including genetic sex-separation, which enables more cost-effective production and release of only male mosquitoes, and a brighter fluorescent marker, which enables field monitoring in all life stages of the mosquito. The key similarities and differences between OX513A and OX5034 are highlighted in the table below.

Technology Characteristics	1 st Generation (OX513A)	2 nd Generation (OX5034)
Effective mosquito control in field trials, with built-in biosafety	Yes; demonstrated in Brazil, Cayman, Panama	Yes; demonstrated in Brazil
No direct effect on non-targeted species	Yes	Yes
Non-toxic, non-allergenic active and inert ingredients	Yes (tTAV and DsRed2)	Yes (tTAV-OX5034 and DsRed2-OX5034) ¹
No long-term effects on the environment; no chemical residues	Yes	Yes
Tetracycline used for rearing male mosquitoes for release	Yes	No
Genetic sex-separation; reduced costs and complexity	No; manual separation to >99.8% males	Yes; genetic separation to 100% males
Advanced fluorescent marker; visible in all post-egg life-stages	No; only visible in larvae	Yes; in larvae, pupae and adults
Multi-generational pest suppression; expected improvements in efficacy	Only one generation	Yes, multiple but limited number of generations

¹ The DNA sequence of tTAV-OX5034 contains additional features (relative to OX513A) that enable its expression only in female mosquitoes, but the protein sequence is the same (99.4 % identity). The DNA sequence of DsRed2-OX5034 contains additional features that enable brighter expression in all mosquito life stages, but the fluorescent protein domain is the same as in OX513A (98.6% identity). All have the same non-toxic and non-allergenic safety profile.

Letter to Eric Bohnenblust

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Please do not hesitate to contact Nathan Rose or myself if you have any questions regarding this application.

Sincerely,

A handwritten signature in blue ink, appearing to read "Keith A. Matthews". The signature is fluid and cursive, with a prominent initial "K" and "M".

Keith A. Matthews

cc: Nathan Rose, Oxitec Ltd.