[SEQ CHAPTER \h \r 1]

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Washington, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

[DATE \@ "MMMM d, yyyy"]

Keith A. Matthews, Agent
C/O Oxitec, Ltd
Wiley Rein LLP
1776 K St. NW
Washington, DC 20006

Subject: Data Deficiency
OPP Decision Number: 549240
EPA File Symbol: 93167-EUP-E
Product Name: OX5034 *Aedes aegypti*
Application Receipt Date: April 1, 2019
EPA Company Number: 93167
Company Name: Oxitec, Ltd

Dear Mr. Matthews:

The Agency has received and begun its in-depth review of the subject application and has determined that it is incomplete or that further information is needed. This letter is a written notification of those deficiencies and identifies your options under 40 CFR 152.105 and Section 33 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended by the Pesticide Registration Improvement Extension Act (PRIA 3). Your options under 40 CFR 152.105 and section 33 of FIFRA are addressed separately because each involves a different timeframe and set of options for responding to this letter. Please ensure that you consider each of the sections below in determining how and when you respond to this letter.

40 CFR 152.105:

As previously described in a telephone conversation on March 19, 2020, pursuant to 40 CFR 152.105, you are allowed 75 days from the date of this letter ending **June 2, 2020** to provide a response concerning the deficiencies listed in this letter. Your response may include making corrections or additions to complete the application, or notifying the Agency of the date on which you expect to complete the application, or withdrawing your application. If you do not respond to this letter within 75 days or if you respond with a date on which you expect to complete the application but fail to meet that scheduled date, the Agency will treat the application as if you had withdrawn it. Withdrawal concludes the Agency's review of your

application. Any subsequent submission of the same application must then be submitted as a new application with a new deadline for EPA to make a determination on your application and subject to a new registration service fee.

The deficiencies identified in the Agency's review at this time are:

1. The nominal concentration of the tTAV-OX5034 protein in homozygous adult OX5304 males must be recalculated using the combined total of the protein band representing the tTAV-OX5034 protein and the Hsp70 protein. Once translated, the tTAV-OX5034 protein is thought to be cleaved at the UBQ-tTAV junction to release the active form of the tTAV protein which, once dimerized, will initiate the positive feedback loop characteristic of the Tet-OFF gene circuit. Consequently, three protein variants are expected to be present in tTAV-OX5034-expressing mosquitoes: tTAV-OX5034, monomeric tTAV (cleaved), and dimerized tTAV (cleaved).

   Because the Hsp70, tTAV-OX5034, and dimerized tTAV proteins are all expected to be around the same molecular weight (size), and because the Oxitec co-incubated with the VP16 (which detects tTAV) and the Hsp70 antibody at the same time, it is theoretically possible that the "Hsp70"-designated protein band consists of one or more of these three proteins. By only reporting on the one, clearly distinguishable tTAV band at around 50 kDa, the study may have therefore underestimated the total tTAV-OX5034-associated protein amount.

2. Oxitec must revise the upper certified limits based on recalculation of the active ingredient above.

3. Oxitec must submit arbovirus testing data utilizing an RT-PCR method with demonstrated diagnostic validation, quantitative measures of analytical specificity and sensitivity, in addition to ~~positive and negative~~appropriate controls and sample size that effectively demonstrate that laboratory colonies are arbovirus free (i.e. Dengue virus, chikungunya virus, west Nile virus, Saint Louis encephalitis, eastern equine encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis, Mayaro and Sindbis virusc). A rationale must be provided for detection threshold as well as sample sizes tested. EPA requires testing methods describing the parameters above be submitted now and will require submission of confirmatory data that EPA must review and determine to be acceptable prior to any field release of mosquitoes.

~~3.~~4. The following revisions must be made in the section G experimental protocol:

   - In trial A, if only a single release is conducted then subsequent data are only valid for dispersal measurements. Multiple releases must be conducted to measure efficacy.
   - For trial A, adult release for mosquito dispersal can only be used to support dispersal area subsequently used for trial B adult releases. To support dispersal distance in trial B for the mosquito rearing box method of deployment with eggs, dispersal distance must be measured through egg releases with the mosquito rearing box using the trial A protocol.

- In trial A, if OX5034 mosquitoes or their offspring are trapped at the outer perimeter of the study design (i.e., 400 m), then trials will need to be reconducted with, 1.) a larger perimeter to determine maximum dispersal area, and 2.) a greater than 500 m distance between control and treated trials. If trials need to be expanded, the number of replicate trials needs to be adjusted accordingly to not go over the maximum allowable acreage under the EUP.
- For trial B, the distance between trial locations (i.e., control and treated locations) will be greater than the maximum dispersal distance observed in trial A. If the maximum dispersal is less than 400 m, trial locations will be separated by at least 400 m.
- Oxitec, Ltd. must reference or submit the qPCR protocol that will be used to verify OX5034 mosquitoes and their offspring.
- Persistence monitoring must continue until no OX5034 fluorescent larvae are found for at least two successive generations, which may exceed the eight-week timeframe outlined in the EUP.
- Oxitec, Ltd. must describe how to dispose of OX5034 or fluorescent mosquitoes that have been trapped.
- Oxitec, Ltd. must ensure to the extent possible that mosquito abatement activity will be the same in the treated and untreated areas of the EUP and will be disclosed to EPA with data in the final report at the time of application for a registration.
- Field testing sites cannot directly abut the open ocean or other waterbody if this limits dispersal distance measures.

In addition to the deficiencies listed above, EPA's review identified additional shortcomings, listed below, for the section G protocol. Addressing these shortcomings now will improve the likelihood your application can be granted as requested and in an efficient manner.

- The rationale provided by Oxitec, Ltd. for only testing in Florida does not discuss how only testing in Florida will demonstrate efficacy against distinct populations of *Ae. aegypti* mosquitoes and satisfy EPA's requirement for at least three distinct testing locations. Justification for these points needs to be provided for testing only in Florida whether trial A only or trial A and trial B are conducted in Florida. In a letter dated February 14, 2020, EPA indicated that for large scale release, (i.e., trial B), data were necessary from at least three test locations. This was prior to EPA's understanding that only trial A might be conducted, EPA maintains that data must be collected from at least three distinct testing locations regardless of which trial or combinations is intended to support efficacy for a FIFRA section 3 registration application.
- Oxitec, Ltd. should consider measuring the hatch rate of OX5034 from the mosquito rearing boxes.

Further review of your application and your response to the deficiencies may identify additional deficiencies and you will be so informed.

FIFRA Section 33/PRIA:

This application is also subject to a deadline for making a determination on the application under FIFRA Section 33, Pesticide Registration Service Fees, established under PRIA. The time frame for the Agency to make a determination on this application ends on March 31, 2020. Because the deadline for the agency

to make a determination on this application expires before the end of the 75 days you have to respond to the deficiencies noted above, you have the following three options:

1. **Establish a new due date**. You may work with us to establish a new section 33/PRIA deadline that allows for an appropriate response to the 75 day letter. If you choose this option, you need to contact the Agency not later than **March 26, 2020** to discuss a time frame that allows you to address the deficiencies listed above and the Agency to make a regulatory decision.

2. **Withdraw the application.** Alternatively, you may notify us not later than **March 26, 2020** that you are withdrawing your application. **As noted above, withdrawal concludes the Agency's review** of your application; however, you may resubmit your application after the deficiencies have been addressed. Should you choose to resubmit your application, it would be subject to a new deadline for making a determination on your application and a new registration service fee. Since a fee was paid with this application, the Agency will provide any applicable refund as soon as practicable.

3. **Not respond.** If the Agency does not hear from you by **March 26, 2020**, the Agency in meeting its obligations under section 33/PRIA may issue a determination to not grant your application. While a determination to not grant an application would allow EPA to have met its obligation under section 33 of FIFRA to issue a determination by a specified date, this determination is neither a denial of the application pursuant to section 3(c)(6) of FIFRA or a withdrawal of the application. Thus, the Agency will continue to diligently work on any such application as long as EPA receives a response to a deficiency notice within the 75 days described above.

Please respond to this letter by **March 26, 2020** by contacting Eric Bohnenblust by telephone on 703-347-0426 or by e-mail at [HYPERLINK "mailto:Bohnenblust.eric@epa.gov"] or Alan Reynolds by telephone at 703-605-0515 or by email at [HYPERLINK "mailto:Reynolds.Alan@epa.gov"] during the hours of 7:00 am to 4:30 pm EST with a response and for any questions concerning this letter. When submitting information or data in response to this letter, a copy of this letter should accompany the submission to facilitate processing.

Sincerely,

Alan Reynolds
Team Leader 94
Emerging Technologies Branch (ETB)
Biopesticides and Pollution Prevention Division (BPPD)