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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

March 7, 2022

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

Subject: Experimental Use Permit Amended for 93167-EUP-2 to Allow Releases of OX5034 *Aedes aegypti* in Florida and California
Experimental Use Permit No.: 93167-EUP-2
OPP Case No.: 00295569
Effective Dates: For OX5034 *Aedes aegypti*: Immediately until April 30, 2024 (pesticide applications and associated activities, e.g., post-release monitoring activities)
Quantity Authorized: 2,455,040,000 OX5034 *Aedes aegypti* mosquitoes, containing 0.0222 pounds (10.06g) of tTAV-OX5034.
Acres Involved: 34,760

Dear Mr. Matthews:

On the basis of the information furnished by you, the subject Experimental Use Permit (EUP) under section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) is hereby amended and extended for the release of OX5034 *Aedes aegypti* mosquitoes containing Tetracycline Trans-Activator Variant (tTAV-OX5034) protein and the genetic material (from vector pOX5034) necessary to produce the protein *in vivo*. The EUP, which is authorized only in the states of Florida and California, will evaluate efficacy of release of adult and eggs of OX5034 *Aedes aegypti* mosquitoes against wild *Aedes aegypti* mosquitoes within Monroe County, Florida and Stanislaus, Fresno, Tulare, and San Bernardino counties, California. Shipment and/or use under this permit are subject to the provisions of 40 CFR Part 172.

Prior to shipment and/or use of this material, you must consult with the pesticide regulatory officials of the states in which you will conduct your experimental program and obtain a state permit or license if such is required. Amendment of this Federal permit does not negate the need for permission from individual states. Prior to initiating this experimental program in any state, you are required to notify the lead agency of the states in which you will conduct your experimental program of the scientific testing program (when, where, how much, etc.). Failure to obtain that permission may result in revocation of modification of the EUP.

You must provide notice to the state pesticide regulatory agency at least 72 hours prior to the application of this experimental pesticide product. You must also provide a copy of this authorization letter and the stamped, accepted EUP labeling to all cooperators, participants, and users prior to the initial pesticide application made in accordance with this EUP. The U.S. Environmental Protection Agency (EPA) will notify the relevant EPA Regions of the approval of this EUP by sending copies of this EUP authorization letter and the stamped, accepted EUP labeling to them.

Based on the experimental program submitted, this product may be shipped for use under this EUP to the states of Florida and California for the next experimental period, effective immediately until 4/30/2024. Post-release activities (e.g., data collection and post release-monitoring) may continue afterwards as necessary in accordance with the experimental program and terms and conditions below. The acres and amounts permitted per state are as follows:

State	Maximum Acres	Number of Mosquitoes	Pounds of Active Ingredient (g)
Year 1: March 7, 2022- April 30, 2023			
Florida	2,980	196,878,881	0.0018 (0.81g)
California	15,600	1,030,641,119	0.0093 (4.22g)
Total (Year 2022-2023)	18,580	1,227,520,000	0.0111 (5.03g)
Year 2: April 30, 2023 – April 30, 2024			
Florida	2,980	196,878,881	0.0018 (0.81g)
California	15,600	1,030,641,119	0.0093 (4.22g)
Total (year 2023-2024)	18,580	1,227,520,000	0.0111 (5.03g)
Overall Permit Total	34,760	2,455,040,000	0.0222 (10.06g)

You will also keep records of production, distribution, and performance, and make the records available on request to any authorized officer or employee of the EPA.

Prior to registration under FIFRA section 3, all data requirements must be satisfied.

The labeling submitted in connection with the application for the EUP is acceptable, and a stamped, accepted copy is enclosed for your records. This labeling must be used for all shipments under this EUP and must be in possession of the user at the time of pesticide application. The following acceptable Confidential Statements of Formula (CSF) are on file for this product:

Basic CSF dated: January 17, 2022
Alternate CSF dated: January 17, 2022

You must provide a final report at the conclusion of the experiment, as well as an annual monitoring report as described in condition number 5. The final report shall include all of the items set forth in 40 CFR § 172.8(b)(2). In addition, you must adhere to the following terms and conditions:

1) Releases must not occur within 500 meters from the outer perimeter of 1) wastewater treatment facilities; 2) commercial citrus, apple, pear, nectarine, and peach crops; 3) and commercial cattle, poultry, and pig livestock facilities.

2) Three adult mosquito traps must be placed within 100 m from the outer edge of each potential environmental tetracycline sources (as identified in term #1 above) that are located within 1,000 m of any OX5034 release point. Catch bags will be collected and replaced between daily (maximum frequency) or weekly (minimum frequency). For female *Ae. aegypti* captured in these traps, Oxitec must determine the presence of the genetic cassette (vector pOX5034) in a minimum of 150 adult female *Ae. aegypti* (or the maximum amount of adult female *Ae. aegypti* trapped, should fewer than 150 adult females be trapped) following the standard operating procedures QD-R-00109 or QD-R-00108 (qPCR or endpoint PCR, respectively) once per week. This monitoring must occur throughout the release period and continue throughout the post-release monitoring period, as defined in Term #7

3) Oxitec must conduct continuous weekly monitoring for fluorescent larvae at release sites as indicated in the section G experimental program (section 4.6.5). From the reared field-collected individuals, Oxitec must determine the presence of the genetic cassette (vector pOX5034) in a minimum of 150 non-fluorescent adult female *Ae. aegypti* following the standard operating procedures QD-R-00109 or QD-R-00108 once per month.

4) If Oxitec finds female individuals containing the OX5034 genetic construct surviving to adulthood through the terms outlined in terms #2, #3, or during quality control procedures in SOP GL-BR-00047, it must take the following remediation actions: immediately (i.e., no later than 24 hours) notify EPA and cease releases of all OX5034 mosquitoes, as soon as practicable apply adulticide and larvicide pesticides to the treated area where the surviving females were detected and continue to monitor for the presence of the OX5034 genetic construct in female *Ae. aegypti* until OX5034 mosquitoes are no longer found for at least two successive mosquito generations, a minimum of 10 weeks. EPA may require additional applications of adulticides and larvicides if fluorescent mosquitoes continue to be found in the treated area after the initial detection.

5) Provide an annual report of the monitoring as outlines in terms #2 and #3 and quality control procedures outlined in term #4.

6) If evidence is found of invasive *Aedes* spp. Or arboviruses principally vectored by *Aedes aegypti* becoming established in the UK or production of homozygous OX5034 is moved outside of the UK, colony related testing will be required.

7) As indicated in the section G experimental program (sections 4.6.5 and 7), Oxitec must conduct post-release monitoring until no fluorescent OX5034 mosquitoes have been found for at least two successive generations, a minimum of 10 consecutive weeks.

8) In the event of tropical storms, hurricanes, known advancing wildfires, or other significant natural disasters Oxitec will return Mosquito Rearing Boxes to a secure facility safely under triple containment (with two of the three containment layers being shatterproof) before the disaster is predicted to reach the trial area, if safe to do so. Boxes will be both transported to and stored in the facility under triple containment (with two of the three containment layers being shatterproof) and may be returned to the field sites as live Mosquito Rearing Boxes if/when safe to do so, to enable ongoing mosquito rearing in the boxes and to minimize trial disruption as a result of natural disasters, while ensuring that mosquitoes are only released in the approved trial areas. Boxes may alternatively be disposed of in accordance with the approved disposal procedures (i.e., killed by freezing and then disposed of in general waste).

If you have any questions regarding this permit, please contact Matt Weiner via email at Weiner.Matthew@epa.gov or by phone at (202) 566-1509.

Sincerely,

MICHAEL GOODIS Digitally signed by MICHAEL GOODIS Date: 2022.03.07 14:32:01 -05'00'

Edward Messina Esq., Director
Office of Pesticide Programs

Enclosure