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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Review of the 75-day Letter Response for the Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 *Aedes aegypti* Mosquitoes Decision #549240

FROM: Kara Welch, M.S., Biologist
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division

Digitally signed by KARA WELCH
Date: 2020.04.28 14:54:18 -04'00'

THRU: Eric Bohnenblust, Ph.D., Senior Biologist
Emerging Technologies Branch

Mike Mendelsohn, Branch Chief
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division

TO: Eric Bohnenblust, Ph.D., Senior Biologist
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

ACTION REQUESTED

Previously, EPA summarized the experimental use permit (EUP) protocol submitted by Oxitec, Ltd. to investigate the pesticidal efficacy of *Aedes aegypti* strain OX5034 for female larval mortality of wild mosquitoes to support a subsequent Section 3 submission (Welch 2020). EPA requested clarification and noted deficiencies associated with the EUP in a 75-day letter dated, March 19, 2020. Herein, EPA determines the adequacy of the response provided by Oxitec, Ltd. and classifies the program for the experimental use permit.

SUMMARY

Oxitec, Ltd. has requested an EUP to investigate the pesticidal efficacy of *Ae. aegypti* strain OX5034 for female larval mortality of wild mosquitoes to support a subsequent Section 3 submission. Oxitec, Ltd. plans to release male *Ae. aegypti* strain OX5034 and compare the survival rates to adulthood between treated female larval progeny (those fathered by OX5034 males) and untreated female larval progeny (those fathered by wild males), over-flooding ratio (i.e., the OX5034 male to wild male ratio), and proportion of treated individuals trapped (i.e., mating fraction). Additional metrics will examine OX5034 male dispersal capacity and persistence of the transgene post-release. The request is not to exceed a total of 6,600 over the

two-year period. The site locations include Monroe County, Florida and Harris County, Texas (see Table 3). For a detailed summary of the EUP protocol supplied by Oxitec, Ltd. please see Welch (2020).

After an initial review of the EUP (Welch 2020), EPA asked for clarification from Oxitec, Ltd. regarding various trial parameters, measurements of efficacy, acreage requests, and number of mosquitos/active ingredient used in the trial. In a 75-day letter response (March 23, 2020), Oxitec, Ltd. revised the Section G submission. Subsequent revisions were also requested via email following the initial 75-day letter response during the month of April 2020. With the revisions made by Oxitec, Ltd. to the Section G submission, EPA now concludes that the protocol is acceptable for the EUP (see CONCLUSION section below).

REVISIONS REQUESTED AND REGISTRANT RESPONSE

Here, EPA will note the action requested of Oxitec, Ltd., summarize the registrant response, and opine on the adequacy of the Section G experimental program.

Table 1. Agency identified deficiencies and registrant revisions to Section G protocol

EPA Request	Registrant Request	EPA Response
In Trial A, if OX5034 mosquitoes or their offspring are trapped at the outer perimeter of the study (i.e., 400 meter), then Trials will need to be reconducted with, 1) a larger perimeter, and 2) a greater than 500 meter distance between control and treated Trials.	The Section G was amended to reflect that Trial areas will be separated by at least 500 meters and if mosquitoes are trapped at the perimeter of Trial A, then additional ovitraps will be installed at distances greater than 500 meter from the release site to capture any mosquitoes that may travel further.	The response is acceptable.
Persistence monitoring must continue until no OX5034 fluorescent larvae are found for at least two successive generations.	Monitoring will continue until at least ten consecutive weeks without the presence in ovitraps of OX5034 fluorescent mosquitoes.	The response is acceptable.
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.	Oxitec, Ltd. amended the Section G protocol to include that for efficacy measures, weekly releases will be conducted.	The response is acceptable.
To support dispersal distance in Trial B, dispersal distance must be measured through egg releases with the mosquito rearing box using the Trial A protocol.	Dispersal distances measured from egg releases will be used to support Trial B.	The response is acceptable.

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.	Trial B sites will be separated by 500 meters.	The response is acceptable.
Oxitec, Ltd. must reference or submit the qPCR protocol that will be used to verify OX5034 mosquitoes and their offspring.	The PCR protocol and the SOPs were submitted in Jul 2019 (Oxitec Ltd; MRID 50889427).	The response is acceptable.
Oxitec, Ltd. must describe how to dispose of OX5034 or fluorescent mosquitoes that have been trapped.	Any fluorescent larvae from ovitraps or fluorescent adults from BG traps, once transported to the lab for identification, will be disposed of by freezing at <15 °C for 12 hours or longer, and then disposed of in general waste.	The response is acceptable
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.	Treated and untreated areas of the EUP will be chosen to be as similar as possible, including in terms of any known mosquito abatement activity. Details of any mosquito abatement activity in Trial areas will be disclosed to EPA with data in the final report, at the time of a Section 3 application.	The response is acceptable.
Field testing sites cannot directly abut the open ocean or other waterbody if this limits dispersal distance measures.	Traps may be constrained in terms of direction by available land mass, although every effort will be made to accommodate the distances required. Water bodies such as canals or ponds up to approximately 100m across will be considered not to impact dispersal.	The Agency understands the location of Trial in the Florida Keys may constrain the capacity to conduct a dispersal Trial in concentric rings in all direction without abutting open ocean or bodies of water. The response is acceptable.
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.	No new information was provided.	For the issuance of an EUP, a certain number of Trial locations is not explicitly required. EPA will opine further when and if a Section 3 registration is submitted.

Oxitec, Ltd. should consider measuring the hatch rate of OX5034 from the mosquito rearing boxes	A response was not provided.	The requested information is not required for the EUP. However, for a Section 3 registration, EPA may request data regarding the hatch rates measured in the EUP rearing boxes to inform the final label.

EPA concludes overall that the Section G protocol with the revisions made above is acceptable (see CONCLUSION section below).

TEST LOCATIONS AND AMOUNT OF ACTIVE INGREDIENT

Oxitec, Ltd., revised the acreage allotted per site and by year in the subsequent submission. The below tables supersede Welch (2020). Oxitec, Ltd. proposes to test male OX5034 *Ae. aegypti* mosquitoes in Monroe County, Florida and Harris County, Texas. In total, Oxitec, Ltd., requests 6,600 acres for testing between the years 2020 and 2021. Of this acreage 4,800 will be treated and 1,800 will be control sites. The requested test locations and acres are listed in the applicant provided table (see Table 2).

Table 2. Number of sites, application rates, and replicates for Trials A and B, including the treated acreage and life-stages assessed for both years

Trial	Location*	Number of untreated areas (required)	Number of treated areas - low dose (required)	Number of treated areas - medium dose (optional)	Number of treated areas - high dose (optional)	Max acreage per Trial site	Maximum total acreage	Life stage assessed
Trial A	Florida - Monroe Co.	3	3	3	3	200	4800	Eggs or adults (one life stage only)
Trial B	Florida - Monroe Co.	3	3	3	N/A	80	1440	Eggs only
	Texas – Harris Co.	3	3	N/A	N/A	40	360	Eggs only

Reproduced from Table 3 in Oxitec, Ltd. (2020).
*Note, both or only one location (FL or TX) may be used.

The acreage request per year and per site has changed significantly from Table 1 in Welch (2020). Previously, Oxitec Ltd.'s acreage request totaled 6,600 acres in a one-year period. The revised Table 2 above accounts for two Trial A iterations in Florida over a two-year period and

one Trial B study in one year all totaling 6,600 acres. Additionally, the maximum acreage per trial site for Trial B has decreased (i.e., 40-80 acres, rather than 100 acres per Trial site, see Welch 2020).

Separated by year, the maximum annual treated acreage will not exceed 3,120 acres per year in Florida for both Trial A and B and 360 acres over one year in Texas, for a total of 6,600 acres (see Table 3).

Table 3. Maximum annual treated and control acreage per state for the duration of the EUP.

State	Acres (Trial A + B)
Year 1	
Florida	3,120
Texas	0
Total (year 1)	**3,120**
Year 2	
Florida	3,120
Texas	360
Total (year 2)	**3,480**
Overall Permit Total	**6,600**

Reproduced from Table 5 in Oxitec, Ltd. (2020).

Across 24-month test period, Oxitec, Ltd. projects no more than 1,266,720,00 male OX5034 mosquitos, or 5,193.56 milligrams of active ingredient (i.e., tTAV-OX5034) in the program will be released given the applicant conducts both Trials in Florida but only one Trial B in Texas for a 12-month term (see Table 4).

Table 4. Applicant provided table on number of male OX5034 mosquitoes and amount of active ingredient to be released per year of the EUP

	Male OX5034 mosquitoes/year	mg tTAV-OX5034 protein/year
Florida		
Trial A	9,360,000	38.38
Trial B	499,200,000	2,046,72
Total	**508,560,000**	**2,085.10**
Texas		
Trial B	249,600,000	1,023.36
Total	**249,600,000**	**1,023.36**

Reproduced from Table 4 in Oxitec, Ltd. (2020).
Note, Oxitec has proposed to conduct Trials A and B in Florida for two years, but only one year in Texas

ACREAGE JUSTIFICATION

In Welch (2020), the Agency re-iterated the Oxitec, Ltd. discussion regarding the expected dispersal of OX5034A mosquitos from each release point as 50 meters (i.e., 1-2 acres) and the maximum dispersal as 500 meters (i.e., 200 acres). In further review, EPA notes the maximum anticipated dispersal of OX5034 is 200 meters which is consistent with numerous other observations of *Ae. aegypti* dispersal including previous field releases of OX5034 in Brazil

where maximum dispersal distance of OX5034 was recorded at 198 meters. Although longer dispersal distances for *Ae. aegypti* have been observed, a compilation of release recapture studies around the world found that most *Ae. aegypti* are recovered within 20 meters to 50 meters of the release point, with a small percentage found 170 meters but generally not more than 200 meters from the release point (OECD 2018).

Thus, a 200-acre site size for Trial A is justified as a conservative upper bound of maximum dispersal for mosquitos and for a study with three replicates of the untreated control, low dose, medium dose, and high dose scenarios, a minimum of 2,400 treated acres are needed per year for Trial A in Florida (see Table 4).

For, Trial B, where multiple release points are being studied and trials are separated by mean dispersal, 40-80 acres is a reasonable and conservative upper bound estimate of mean dispersal.

For both trials, a conservative restriction of 500 meters between control and treated sites (more than the estimated 200 meter maximum dispersal for released OX5034 males + 200 meters for mated *Ae. aegypti* females) will be implemented.

CONCLUSION

EPA finds the Section G protocol submitted by Oxitec, Ltd. is acceptable for the purpose of an Experimental Use Permit. However, whether the amount of data collected by the proposed protocol will support a FIFRA Section 3 registration application will depend on additional rationale or justification submitted by Oxitec Ltd. to inform EPA's decision on which efficacy measure EPA determines to be valid for measuring efficacy of OX5034 as well as justification for how a single Trial A study will be representative of distinct mosquito populations (see Table 1 and refer to Welch (2020) for a detailed summary of the EUP protocol provided by Oxitec, Ltd.).

REFERENCES

OECD. 2018. Safety Assessment of Transgenic Organisms in the Environment, Volume 8.

Oxitec, Ltd. 2020. Application for experimental use permit to use OX5034 *Aedes aegypti* males for mosquito control. Section G. MRID Unassigned. Submitted to EPA on April 24, 2020.

Welch, K. 2020. Review of Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 *Aedes aegypti* Mosquitoes. Memorandum from K. Welch through E. Bohnenblust to E. Bohnenblust and M. Mendelsohn. Dated April 02, 2020. United States Environmental Protection Agency.