Follow-up to FKMCD-Oxitec July 28, 2020 Public Educational Webinar

Event Summary, List of Questions Asked and Answered, and Additional Resources

August 4th, 2020

FKMCD and Oxitec held a public educational webinar on July 28, 2020 at 5pm ET. The following is a summary of the event, questions asked and answered, answers to questions submitted after the event, and additional helpful resources for topics discussed.

Event Summary:

- **A complete recording of the event can be viewed here**
- **The event explored Oxitec technology and was entitled '*From Lab to Field: Why it Works and Why it's safe*'.**
- **The event was moderated by Meredith Fensom (Oxitec, Head of Public Affairs), and presenters were Dr Neil Morrison (Oxitec, Head of Agriculture Programs) and Dr Nathan Rose (Oxitec, Head of Regulatory Affairs).**
- **The event lasted 60 minutes, devoting half of the time for Q&A.**
- **19 questions were individually answered, questions were not batched together.**
- **Questions were answered anonymously to ensure attendees were not inhibited by disclosure of their names.**

Title: From Lab to Field: Why it Works and Why it's safe.

Date: July 28th, 2020

Panelists: The event featured the following panelists:

Moderator: Meredith Fensom (Oxitec, Head of Public Affairs)

Presenter: Dr Neil Morrison (Oxitec, Head of Agriculture Programs)

Presenter: Dr Nathan Rose (Oxitec, Head of Regulatory Affairs)

Question and Answer Catalogue: the following provides details of the 20 questions asked and answered, and additional information resources.

Topic for Easy Reference	Questions Asked	Answers	References
Questions About Regulation, Oversight			
Level of regulation / under-regulation of Oxitec's mosquito technology	*"Why do you feel that a 2-page marketing memo and only an Environmental Assessment is sufficient level of evaluation?"*	When providing information about an EUP for public comment, the EPA is required by 40 CFR 172 to provide certain information to the public. EPA complied with the relevant regulation when opening public comment on the Oxitec OX5034 EUP, and described the information as follows (p92 of EPA's Response to Comments): "For an EUP notice of receipt (NOR) EPA customarily provides the following information: the name of the pesticide, the name of the submitter, purpose of the EUP, the maximum application rate and use site, maximum number of treated acres requested, duration of EUP, and location of test site(s). In addition to that information, EPA provided the public a summary of the key differences between the first generation OX513A mosquitoes and this second-generation product (0002) as described in Unit I of this Response to Comment document. Further, the EUP regulations regarding "Publication" at 40 CFR 172.11(a) state, in part: (a) Notice of receipt of an experimental use permit application. The Administrator shall publish notice in the FEDERAL REGISTER of receipt of an application for an experimental use permit upon finding that issuance of the experimental use permit may be of regional or national significance. This notice shall include: (1) The active ingredients, (2) Use pattern(s),	EPA's full regulatory package. State of Florida findings.

		(3) Quantity of pesticide, (4) Total acreage, (5) Location of area of application, (6) A statement soliciting comments from any interested persons regarding the application. Here, EPA published a Notice of Receipt (NOR) of the EUP application in the Federal Register, in compliance with 40 CFR 172.11, soliciting public comment for 30 days, upon a finding that issuance of the EUP may be of regional or national significance. 84 Fed. Reg. 47,947 (Sept. 11, 2019). The NOR and public comment period provided fulfill the requirements of the "publication" regulations." EPA followed the same procedures when opening public comment periods on the Wolbachia-infected mosquito technology, providing the same information required by 40 CFR 172. Regarding the risk assessment of the EUP, EPA followed the relevant FIFRA requirements when assessing the EUP application for the OX5034 mosquito.	
Oxitec Peer-Reviewed Papers	*"Are all the peer-reviewed papers available for the public to read, if so, where can we find them. Also, is there an index for easy reference by subject?"* *"Oxitec claimed all of those papers you showed earlier were Independent reviews, but the first name I looked at was Derric Nimmo [a former*	The list of Oxitec's peer-reviewed publications is available on the company's website: https://www.oxitec.com/en/our-technology by scrolling to the bottom of that page to the section headed 'Scientific Publications.' Many, but not all, of the papers are 'open access' and can be freely accessed by clicking on the links provided. Some papers are behind journal paywalls which require subscriptions to access the publications. If you would like to access a specific publication, please email florida@oxitec.com and Oxitec will endeavor to provide a copy of the publication (may be subject to copyright restrictions). The peer review process works as follows:	

	Oxitec staff member]. How is this independent?"	1. Scientists carry out experiments and write a journal article describing the results, listing themselves as authors. 2. The journal editors send the article and its supporting data to several carefully selected peer reviewers (usually 3-5 reviewers) who are independent scientists and experts in the field, i.e. not connected to the article authors in any way. Peer reviewers are usually anonymous, and their identities are not typically revealed to the article's authors. 3. Peer reviewers give feedback on the article, focusing on whether the experiments have been correctly carried out, whether the data analysis is appropriate for the type of data, and whether the conclusions are correct based on the data. 4. The editor gives the feedback to the article authors, with instructions to amend or correct the article if required. 5. If the amendments are satisfactory (and this may require the reviewers to re-review the article after amendment), then the journal may accept the article for publication. Therefore, articles published by Oxitec in peer-reviewed journals will always have Oxitec staff scientists listed as authors on those articles. The names listed on the articles are the scientists who carried out the work, not the peer reviewers.	
FKMCD Board Oversight	*"Is this webinar just Oxitec people talking, or can we ask board members questions?"*	Comments to FKMCD Board members are available at public board meetings.	

Questions About the Technology			
Tetracycline usage and antibiotic-resistant bacteria	*"Tetracycline is controversial here in the Keys, what did the regulators decide?"* *"In an earlier webinar, I believe the amount of tetracycline used to produce mosquitoes for the Keys would less than a sugar packet - today you said there would be "no tetracycline in deployments" - can you explain the role of tetracycline in this process?"* *"Why not test eggs for antibiotic resistance?"*	**Oxitec will not be using tetracycline in Florida**, and the eggs shipped to Florida will have **never been in contact with tetracycline**. There is no risk and thus no scientific basis for testing. The EPA, FDA and Florida regulators looked at this exhaustively and found no risk. No exposure of Oxitec male mosquitoes to tetracycline, either as eggs in the UK or as adults in the US, means no potential for selection of resistant bacteria. The entire production process was reviewed and validated by the EPA and state regulators. Dr. Nathan Rose provided a detailed overview of Oxitec's production process and how **tetracycline is used in the UK**, and how Oxitec's mosquitoes being used in Florida will not be in contact with tetracycline. He highlighted that a small amount (less than a sugar packet, or approximately 5 g) of tetracycline will be used to manage the OX5034 colony **in the UK**, but all eggs from that process are surface-sterilized with a sanitizing agent 4x the strength of hospital-grade disinfectant before being shipped. **No tetracycline is used to produce male adult mosquitoes in Florida, which will be deployed in the field**. He also noted that Oxitec responds readily to any data requests issued by regulators but does not respond to *ad hoc* requests for data made by private individuals.	EPA: "negligible risk that testing of OX5034 mosquitoes would spread antibiotic resistant bacteria in the US environment" *(p75-76, Response to Comments).*
Genes used in the OX5034 mosquito	*"Could you explain again how you make sure it is a female only effect?"*	The tTAV protein is produced in large quantities inside cells in the developing female mosquito. It blocks the cells from carrying out normal cellular processes and from producing many of the other proteins required for normal mosquito development. This stops the female larvae from developing to pupae and adults, and they die as early-stage larvae. The action	

		of the tTAV protein can be blocked by tetracycline-class antibiotics if used at the right concentrations.	
Male mosquitoes biting	*"What is the advantage of letting the males survive?"*	Male mosquitoes cannot bite. They lack the mouthparts to do so. No female Oxitec mosquitoes will be released during this project. Surviving male mosquitoes can pass on the self-limiting gene to their offspring. Female offspring that inherit the gene will die, while male offspring will survive and can pass on the gene again.	
Introgression of background genes	*"Is there a way to reduce the lifespan of male Oxitec mosquitoes to a sweet spot where they mature enough to mate, but don't live long enough for introgressing to potentially be an issue?"*	Because transfer of genes (including the Oxitec self-limiting gene) occurs via mating, there is no way to separate mating from introgression. However, **EPA found no scientific grounds for concern about introgression, nor did the CDC.** In EPA's review of the data, they noted that "introgression of OX5034 strain genetics into the local wild *Ae. aegypti* mosquito population is likely to occur during releases of OX5034; however, the risk resulting from such introgression is negligible" (p134, EPA Response to Comments). Hybrids would have increased susceptibility to insecticides, making them easier to control, and no enhanced ability to spread disease is expected. "In conclusion, given the data on insecticide resistance, longevity, and fecundity, the large impact of the environment on all traits evaluated, and the complexity of vector competence, EPA believes it is unlikely that the introgression of OX5034 strain genetics would result in increased vectoral capacity of the local mosquito populations under the applied for EUP." (p40, Human Health and Environmental Risk Assessment).	EPA Human Health and Environmental Risk Assessment EPA Response to Comments

Female release	*"Why did Dr Gorman say you were allowed to have so many females and from your EPA documents only 500 eggs were tested for females? That doesn't seem like a statistically significant sample size given 500M."* *"Oxitec's initiative depends on citizen support. If GM Female mosquitos are detected, will you shut down your trial/experiment?"* *"If a female bred with OX5034 and laid eggs in an environment with tetracycline, could females survive?"*	**Zero females will be released with OX5034, as the new strain is male-selecting, female-lethal.** OX5034 does not allow for female survival, and thus no females will be released. These data have been reviewed by EPA and Florida state regulators: "exposure to female mosquitoes … was determined to be negligible given that the penetrance of the tTAV-OX5034 lethal trait was shown to be 100% in female mosquitoes" (p50, Human Health and Environmental Risk Assessment). The question referring to '*500 eggs tested for females*' does not match any of the data supplied by Oxitec to EPA, and it's not clear what the basis of this question is. Experimental data (from both lab and field trials) demonstrating effectiveness of the OX5034 self-limiting gene in killing females used sample sizes that were scientifically and statistically appropriate, and all data were reviewed and approved by EPA and Florida state regulators. Ongoing quality control of all egg batches also ensures the ongoing effectiveness of the self-limiting gene in killing female OX5034 progeny. In the unlikely event of a female bred with OX5034 laying eggs in an environment with tetracycline present, then female OX5034 mosquitoes could survive if the growth conditions were appropriate and if the tetracycline concentration were high enough. However, EPA assessed this possibility: "*Several lines of evidence including a survey of environmental levels of tetracycline, tetracycline dose-response testing of OX5034 females, and oviposition behavior of Ae. aegypti, indicate that the risk of hemizygous OX5034 female mosquitoes emerging in the environment*	The U.S. EPA's approval of Oxitec's proposed pilot project. EPA's Human Health and Environmental Risk Assessment.

		due to high levels of tetracycline is low. Trial site location restrictions using known Ae. aegypti dispersal distances to limit exposure to locations with higher probabilities of containing tetracycline would further reduce the likelihood of OX5034 females in the environment to the point where the risk would be considered negligible." This is a summary of a much more extensive discussion of this issue, which is available on p31-34 of the Human Health and Environmental Risk Assessment. If female OX5034 mosquitoes were to be detected during the project, EPA has prescribed specific steps to be followed: *"If at any time during the course of the EUP Oxitec finds female individuals containing the OX5034 genetic construct surviving to adulthood Oxitec must take the following remediation actions: immediately 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."* (EUP Issuance Letter, EPA).	
Persistence in the field	*"How does your mosquito disappear after a few generations if males survive (to breed with wild females)?"*	Released males will be homozygous for the self-limiting gene (i.e. they have two copies of the self-limiting gene). When they breed with wild females, all the offspring will inherit one copy of the self-limiting gene, and females will die. Surviving males, with one copy of the self-limiting gene, will pass on the gene to half of their offspring, and any females inheriting the gene will die. In	

		the subsequent generation, one-quarter of the offspring will inherit the gene, one-eighth in the generation after that, and so on until the gene disappears from the environment. This is because the self-limiting gene obeys normal Mendelian inheritance laws. This is expected to occur within 7-10 generations after the release of the original homozygous male OX5034 mosquitoes, and field data from Brazil have confirmed this. EPA also confirmed this, stating "*Therefore, upon cessation of the proposed OX5034 male releases, it is expected that the OX5034 transgene would disappear from the environment within 10 generations*." (p39, Human Health and Environmental Risk Assessment).	
Malaria, other vectors	*"Would the technology for creating the genetically modified Aedes aegypti theoretically function for other species as well, notably Anopheles gambiae? Are there projects underway in Africa to address Anopheles gambiae/malaria?"*	Yes. Oxitec has development programs underway, funded by the Bill and Melinda Gates Foundation, to develop the same technology in two malaria vectors, *Anopheles stephensi* and *Anopheles albimanus*. Oxitec does not currently work on *Anopheles gambiae,* but the self-limiting genes would likely work in the *Anopheles gambiae* species complex too. The Target Malaria project (not connected with Oxitec) is presently attempting to develop a solution for the *Anopheles gambiae* malaria vector.	https://www.oxitec.com/en/our-technology https://targetmalaria.org/
Nuisance mosquitoes	*"Are there any discussions underway to deploy Oxitec mosquitoes in regions with mosquito-dense populations that do not carry serious disease?" Not as serious an issue as in countries with Zika or malaria, but*	Oxitec's self-limiting technology is likely to be transferrable to other mosquito species beyond *Aedes* and *Anopheles*, but there are currently no plans to do so.	

	still a major nuisance.		
Questions About the Project Location, Environment and COVID			
Impact on ecosystem and endangered species	*"How do you know this technology won't affect other insects and also animals that may feed on them"*	**Oxitec mosquitoes will not have a negative impact on the Keys' ecosystem, or any effect on endangered species**. Oxitec's non-chemical approach is targeted to the invasive *Aedes aegypti* mosquito only and will have no effect on beneficial insects, animals, plants, soil, water, or other parts of the ecosystem. Oxitec commissioned third-party scientists to study the effects on mosquito predators (freshwater fish and invertebrates) of ingesting OX5034 mosquito larvae and pupae, compared with a diet of non-GM mosquito larvae and pupae. No adverse effects on predators were observed as a result of consumption of OX5034 mosquitoes. EPA and FDACS reviewed these data as part of their environmental risk assessment (p43-49, Human Health and Environmental Risk Assessment). *Aedes aegypti* invasive mosquitoes also do not form a major part of the diet of any species in the Florida Keys ecosystem, whether birds, bats, fish, amphibians and reptiles, invertebrates, etc.	EPA: "no adverse effects are anticipated for nontarget organisms as a result of the experimental permit to release OX5034 mosquitoes" (p 49, Human Health and Environmental Risk Assessment). With regard to endangered species, **EPA made a 'No Effect' determination for direct and indirect effects to federally listed endangered and threatened species, and for their designated critical habitats** (p 49, Human Health and Environmental Risk Assessment).
	"Your technology seems strong, supported by robust science and regulatory oversight. Can you name ANY possible danger to humans, flora or fauna in our environment as a result of your technology, and if not, why are many people in the Keys resistant to your project?"	The approval of this project by EPA and Florida state regulators confirmed that there would be no danger to humans, flora, or fauna in the Florida Keys environment due to the releases of OX5034 male mosquitoes. EPA stated "*Since only male mosquitoes will be released into the environment and they do not bite people, they will not pose a risk to people. It is also anticipated that there would be no adverse effects to animals such as bats and fish in the environment*."	EPA statement approving Oxitec's EUP.

		Approximately 1 billion Oxitec mosquitoes have been released over 10 years in 4 countries representing 3 continents. Not one single adverse effect on environmental or human health has ever been documented. The real danger to Keys residents is the presence of *Aedes aegypti* mosquitoes which can transmit disease, as seen in the current local outbreak of dengue fever in Key Largo.	
Number of mosquitoes	*"You can release 1.3Billion mosquitoes according to the test review and amendment document from the EPA. When do you get to release 1.3B vs 500M?"*	The purpose of releasing male Oxitec mosquitoes is to release enough male mosquitoes to find and mate with as many wild females as possible. The number of males released may therefore vary depending on the wild population. The figures quoted in this question relate to the total maximum number of mosquitoes authorized by EPA for two years of releases in both Florida and Texas and are calculated from the maximum weekly release rates permitted. EPA has determined a maximum release rate which may not be exceeded, which is up to 20,000 non-biting males per acre per week. Oxitec does not expect to release at the maximum rate, having achieved successful population suppression in Brazil with far lower mosquito release rates.	EPA Human Health and Environmental Risk Assessment of OX5034
Location	*"Why doesn't Oxitec conduct the experiment in an area where there is no human population?"*	*Aedes aegypti* is a mosquito that has evolved to live near humans and is not found in unpopulated areas. Therefore, the releases will take place in populated areas, where *Aedes aegypti* are found and are able to transmit diseases to humans.	