Follow-up to FKMCD-Oxitec August 11, 2020

Public Educational Webinar

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

August 14th, 2020

FKMCD and Oxitec held a public educational webinar on Aug 11, 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 was entitled '*Oxitec's Vector Control Performance – Past, Present and Future.*'**
- **The event was moderated by Meredith Fensom (Oxitec, Head of Public Affairs), and presenters were Andrea Leal (Executive Director, FKMCD), Dr Kevin Gorman (Oxitec, Head of Field Operations) and Dr Nathan Rose (Head of Regulatory Affairs, Oxitec).**
- **The event lasted a little under 60 minutes, devoting half of that time to Q&A.**
- **7 questions were individually answered during the webinar. As announced at the beginning of the webinar, questions were batched together where appropriate, and questions that had been answered in previous webinars were not repeated. However, repeated questions are included here, together with answers that have previously been provided.**
- **Questions were answered anonymously to ensure attendees were not inhibited by disclosure of their names.**

Title: Oxitec's Vector Control Performance – Past, Present and Future.

Date: Aug 11th, 2020

Panelists: The event featured the following panelists:

Andrea Leal
Executive Director
FKMCD

Meredith Fensom
Head of Public Affairs
Oxitec

Kevin Gorman
Head of Field Operations
Oxitec

Nathan Rose
Head of Regulatory Affairs
Oxitec

Question and Answer Catalogue: the following provides details of the 7 questions asked and answered, and additional information resources.

Topic for Easy Reference	Questions Asked	Answers	References
Questions About Regulation, Oversight			
Regulatory Oversight	*Does the EPA agree with how you will review your data?*	Oxitec's Field Protocol (also known as 'Section G' of the EUP Application) included full details of all data analysis to be conducted when analyzing the proposed project. EPA and FDACS reviewed and approved the Field Protocol, including the data analysis metrics, as part of the EUP approval at both federal and state levels.	EPA's full regulatory package. Section G Field Protocol EPA Review of Section G EPA Review of Section G (Addendum) State of Florida findings.
Questions About the Technology			
Genes used in the OX5034 mosquito	*"I heard that E coli and herpes virus DNA was used to create Oxitec mosquitoes. Should it scare people? If not, why not?"*	The mosquitoes do contain synthetic DNA sequences not found in nature, but which are based on naturally occurring DNA sequences found in a number of organisms. This is not scary in any way. The gene products (proteins) are safe, non-toxic and non-allergenic. (p5, p12, EPA Human Health and Environmental Risk Assessment).	
Persistence in the environment	*"Do Oxitec mosquitoes persist in the environment? Do they pose any risk to endangered species here in the Keys?"* *"When Oxitec leaves, won't the mosquitoes remain in the Florida Keys forever?"*	Oxitec's mosquitoes will disappear from the environment rapidly after releases stop. 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	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**

		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 in less than 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). **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.	**their designated critical habitats** (p 49, Human Health and Environmental Risk Assessment).

Tetracycline	*"Can you please explain again if there is any risk associated with tetracycline? Do the EPA, FDA or state regulators think this is a risk of any type?"* *"Has Oxitec done studies in the Keys about the availability of Doxycycline especially in wastewater, if so what was the outcome?"* *"Is Oxitec aware that oxytetracycline is utilized in widespread agricultural applications in citrus groves throughout Florida to prevent 'citrus greening'?"*	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. 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*	The U.S. EPA's approval of Oxitec's proposed pilot project. EPA's Human Health and Environmental Risk Assessment.

		mosquitoes emerging in the environment 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. EPA has also included restrictions on the project locations: releases will not be carried out within 500 m of citrus groves (where oxytetracycline could potentially be used for control of citrus greening) or within 500 m of municipal wastewater treatment plants (where pharmaceutical-use doxycycline might be present in effluent) (see EPA's approval of Oxitec's proposed pilot project). 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).	
Previous Trial Data	*"Kevin Gorman referred to the math to analyze suppression as simple. Oxitec still has not answered why the [Cayman] MRCU data shows a maximum of 61% suppression yet Oxitec used questionable scientific protocol to calculate 96% suppression?"*	All field performance data, and the parameters that define published metrics, are reported transparently. A range of metrics can be and are utilized to appropriately suit their specific context. In the case of Cayman 2016, where the peak suppression value published in the annual report was 62%, MRCU and Oxitec formally agreed upon a 7-week wet-season average (mean) using eggs per trap as the metric. Other formulas could be used or averaged over a different time period. For example, means calculated over shorter periods result in higher numbers (as the resulting analysis is 'spikier') and those calculated over longer periods result in lower numbers (as they 'smooth' out the analysis).	Annual report MRCU - June 2017 (Friendly Aedes aegypti project in West Bay).
Questions About the Project Location, Environment and COVID			
Caged trials	*"Why are you not doing caged trials in Florida? A large caged trial that mimics the actual Florida environment would help demonstrate how the trial would work without releasing the GE mosquitoes into the environment before careful review. You could have looked at issues of introgression. You did this in your Diamond Back*	**The proposed project has undergone careful review from federal and state agencies prior to approval.** **With regard to Oxitec's caged trials in India,** India is unusual in that its regulators requested this as part of the regulatory process. Most other countries don't have this requirement, including the USA. In the same way as it assesses other mosquito control technologies, including *Wolbachia*, the EPA assessed potential impacts on humans and the environment in permitting open field releases of Oxitec's mosquitoes, considering completed and validated evaluations of the strain in contained and open field settings.	p.134, EPA Response to Comments. p. 40, EPA Human Health and Environmental Risk Assessment. Data published from Oxitec-Cornell Diamondback Moth trials in New York State.

	Moth review in New York State."	Further, **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). "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). The Oxitec-Cornell Diamondback Moth trials in New York State included both caged field trials and open field releases, authorized by USDA. Data from those trials was published in a peer-reviewed journal in early 2020.	
Trial results	*"How Will Oxitec and FKMCD share the results of the trial?"* *"Will the FKMCD staff be part of data evaluation? Who else will be involved in reviewing and evaluating the data?"* *"Does Oxitec have control of the*	If the project is approved by FKMCD, the project has substantial independent review built-in, including by an Independent Advisory Board (Florida Department of Health, University of Florida, local veterinary specialist), CDC specialists, regulators at the state and federal level, and FKMCD themselves. As a team, and subject to Steering Committee approval, we intend to publish all novel scientific findings in peer-reviewed scientific journals, constituting further independent review. We always aim to publish in open access journals, so the data become publicly available for free.	

	release of data and results of the project field performance as they did before in 2016, or will data be available for review unfiltered by Oxitec? Will it be public record?"		
COVID-19 considerations	*"Have project plans been adjusted to reflect the FKMCD board's desire to see the project not interfere with COVID?"* *"How can you justify continuing with this experiment in the middle of a pandemic when at the Mosquito Boards last meeting on July 21, they postponed their vote on this release until Aug 18 due to the high Covid19 numbers, which were 935 on that day and cases have now risen to 1548 and still climbing?"*	The FKMCD Board postponed their vote last month by 30 days to examine in more detail the interaction between the project and COVID. Should approval of the project by FKMCD Board be forthcoming, releases would not begin before 2021 to minimize any impact. Operations would be carried out sensitively with staff and public safety at the forefront of any decisions. The current proposed agreement is available from the FKMCD website.	FKMCD website.
Questions about Oxitec Mosquitoes and Conventional Mosquito Control			
Resistance, chemical applications	*"Resistance to organophosphate applications of pest control is a very real concern. Which chemical applications will be utilized during the*	All FKMCD operations outside of the proposed project will continue as normal. That relates to both vector surveillance and applications to control a range of species, including nuisance biting mosquitoes and disease vectors.	

	experimental trials? What will the frequency of those applications be? How is that different from simply utilizing the chemical applications alone? How will Oxitec determine the success of the experiment when chemical applications will continue throughout the experimental trial?"	FKMCD will endeavor to treat all areas of the proposed project in the same manner, to prevent any bias. All treatments applied are recorded and formally reported to the regulators. They will also be considered during the interpretation of results. This is the same as for prior Oxitec projects in other countries and would typically be the same for FKMCD projects with other technologies.	
Comparison to chemical pesticides	*"How does Oxitec's performance measure up to chemical pesticides? What are the benefits of Oxitec over traditional methods?"*	All vector control technologies have utility and the appropriate choice of product should be evaluated on a case-by-case basis. In general terms, the levels of control achievable with biological mating-based solutions such as Oxitec's can be very high, as males are adept at finding females even when they are few in number. Indeed, as explicitly covered in the webinar content 90+% *Aedes aegypti* population suppression has been documented by Oxitec in peer-reviewed academic journals and elimination of *Aedes aegypti* populations in closed cages (i.e. with no immigration) has also been demonstrated. Contrastingly, it is not uncommon for insecticides to be most efficient when pest populations are high, as then the chemical applications can contact many more mosquitoes. FKMCD typically experiences control of *Aedes aegypti* with chemical treatments of less than 60%.	FKMCD-Oxitec Webinar 4