



OXITEC

Follow-up to FKMCD-Oxitec Sept 30, 2020 Public Educational Webinar #6

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

Oct 20th, 2020

FKMCD and Oxitec held a public educational webinar on Sept 30, 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 '*Environmental Health and Oxitec: Benefits for the Florida Keys' Sensitive Ecosystem and Endangered Species.*'
- 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 approximately 60 minutes, devoting half of that time to Q&A.
- 17 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: Environmental Health and Oxitec: Benefits for the Florida Keys' Sensitive Ecosystem and Endangered Species.

Date: Sept 30th, 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 17 questions asked and answered, and additional information resources.

Topic for Easy Reference	Questions Asked	Answers	References
Questions About Regulation, Oversight			
Regulatory Oversight	<p><i>Question for the panel: In your vast experience in this field, Which hurdle is more rigorous: An 'independent' scientific evaluation' OR 'EPA' approval of an experimental use permit?</i></p>	<p>The EPA assessment process of the OX5034 mosquito, governed under the formal FIFRA and PRIA processes, was exhaustive, evaluating over 4000 pages of scientific data and peer-reviewed scientific literature.</p> <p>It should be noted that an EPA approval of an Experimental Use Permit is indeed a robust independent scientific evaluation.</p>	<p>EPA's full regulatory package.</p> <p>Section G Field Protocol</p> <p>EPA Review of Section G EPA Review of Section G (Addendum)</p> <p>State of Florida findings.</p>
Questions About the Technology			
Persistence in the environment.	<p><i>Can you explain how the two genes in Oxitec mosquitoes do not persist in the environment?</i></p> <p><i>Some consider this synthetic genetic pollution of our ecosystems. What long term evolutionary testing and off target mutation analysis can you provide to show it will never have negative consequences on our environment?</i></p>	<p>Oxitec's mosquitoes will disappear from the environment rapidly after releases stop.</p> <p>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 in less than 10 generations after the release of the original homozygous male OX5034 mosquitoes, and field data from multiple field trials in Brazil have confirmed this.</p>	<p>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).</p> <p>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).</p> <p>EPA Response to Comments</p>

		<p>EPA also confirmed this, stating “<i>Therefore, upon cessation of the proposed OX5034 male releases, it is expected that the OX5034 transgene would disappear from the environment within 10 generations.</i>” (p39, <u>Human Health and Environmental Risk Assessment</u>).</p> <p>Furthermore, EPA found no scientific grounds for concern about introgression, nor did the CDC.</p> <p>In EPA’s review of the data, they noted that “introgression of OX5034 strain genetics into the local wild <i>Ae. aegypti</i> mosquito population is likely to occur during releases of OX5034; however, the risk resulting from such introgression is negligible” (p134, EPA <u>Response to Comments</u>).</p> <p>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, <u>Human Health and Environmental Risk Assessment</u>).</p>	
<p>Previous field trial data</p>	<p><i>In Brazil it was found that the OX513A started failing 18 months into the release program, one likely issue was discrimination by females toward the GM males. Why</i></p>	<p>This is an incorrect statement, and likely originated from similar false statements made by the authors of a paper published in <i>Scientific Reports</i> in 2019 (Evans <i>et al.</i> Transgenic <i>Aedes aegypti</i> Mosquitoes Transfer Genes into a Natural Population. <i>Scientific Reports</i> Vol 9, Article number: 13047 (2019)). This paper was later subject to an Editorial Expression of</p>	<p>https://www.nature.com/articles/s41598-020-62398-w</p>

	<p><i>would you expect this not to occur?</i></p>	<p>Concern which highlighted many problematic issues in this paper.</p> <p>The paper to which this statement refers (Garziera, L., Pedrosa, M.C., de Souza, F.A., Gómez, M., Moreira, M.B., Virginio, J.F., Capurro, M.L., and Carvalho, D.O. (2017). Effect of interruption of over-flooding releases of transgenic mosquitoes over wild population of <i>Aedes aegypti</i>: two case studies in Brazil. <i>Entomol. Exp. Appl.</i> 164, 327–339) shares four authors with the Scientific Reports paper in question, and those same authors make no such conclusion in this earlier paper describing the suppression program in Bahia state, Brazil. In fact, (Garziera et al., 2017) states that mosquito populations in the two treated areas remained suppressed for some time after OX513A releases ceased: <i>“The mosquito population in Juazeiro (Mandacaru) remained suppressed for 17 weeks after the release interruption, whereas in Jacobina (Pedra Branca) suppression lasted 32 weeks.”</i> There is no evidence in (Garziera et al., 2017) to support speculation that the program started to break down while OX513A mosquito releases were under way.</p> <p>Further, there is no evidence to support the assertion that discrimination by females occurred against the GM males, which is directly contradictory to the published data in the paper.</p> <p>Oxitec has released approximately 1 billion mosquitoes over the last decade, and discrimination of wild females against GM males has never been observed.</p>	
<p>Effects on non-target organisms</p>	<p><i>If a lizard or fish eats Oxitec mosquito larvae, what happens to the lizard or fish? Why doesn't</i></p>	<p>Oxitec mosquitoes will not have a negative impact on the Keys’ ecosystem, or any effect on endangered species.</p> <p>Oxitec’s non-chemical approach is targeted to the invasive <i>Aedes aegypti</i> mosquito only and will have no effect on beneficial</p>	

	<p><i>Oxitec's mosquito have an impact?</i></p> <p><i>What 3rd party labs tested sensitive species and where are the studies, for toxicity and other negative effects due to consumption?</i></p> <p><i>Does Oxitec need to conduct "controlled biological tests" on the endangered species down here who eat them, or can be bitten by those females "that don't exist", to show that the mosquitoes won't have impact them? Why are the many tests that the EPA required of Oxitec sufficient?</i></p>	<p>insects, animals, plants, soil, water, or other parts of the ecosystem.</p> <p>Oxitec commissioned third-party scientists in several European contract research laboratories to study the effects on known 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, and summaries of these studies are available (p43-49, Human Health and Environmental Risk Assessment).</p> <p><i>Aedes aegypti</i> 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. Experiments on endangered species, as suggested by this question, are not required or permitted under EPA's data requirements for biopesticides. Instead, EPA, like other scientific regulatory bodies, mandates tests on appropriately selected model organisms.</p> <p>No female OX5034 mosquitoes will be released, hence any evaluation of the impact of OX5034 mosquito bites on endangered species does not have any relevance.</p>	
<p>Resistance to self-limiting gene</p>	<p><i>Can you explain more about why mosquitoes develop resistance to pesticides? Why don't Oxitec mosquitoes generate resistance?</i></p>	<p>"Natural selection by an insecticide allows some initially very rare, naturally occurring, pre-adapted insects with resistance genes to survive and pass the resistance trait on to their offspring. Through continued application of insecticides with the same MoA, selection for the resistant individuals continues so the proportion of resistant insects in the population increases, while</p>	<p>https://en.wikipedia.org/wiki/Pesticide_resistance</p> <p>https://irac-online.org/about/resistance/</p>

		<p>susceptible individuals are eliminated by the insecticide. Under permanent selection pressure, resistant insects outnumber susceptible ones, and the insecticide is no longer effective.” (IRAC Resistance Definitions, https://irac-online.org/about/resistance/)</p> <p>Resistance to chemical insecticides often occurs via metabolic resistance (insects can detoxify the insecticide more effectively) or target-site resistance (the insecticide target site changes to block the insecticide from binding effectively).</p> <p>Oxitec’s self-limiting gene works very differently to chemical insecticides; it produces a protein (tTAV) which interferes with multiple cellular processes and likely has many binding sites inside the cell, making resistance both extremely unlikely to occur, and likely very costly to the cell’s normal functioning. For these reasons, resistance to the self-limiting gene is extremely unlikely to occur and has never been observed in over a decade of Oxitec mosquito releases, with approximately a billion male mosquitoes released.</p>	
	<p><i>Are you worried that the Brazilian biologist Warwick Kerr’s experience in 1950 will happen again? He crossed the African bee with native European bees and the result was an aggressive and difficult to control. And they wiped out the native bees.</i></p>	<p>The development of bee hybrids by crossbreeding the East African lowland honeybee with European honeybee species, is an oft-cited example of unintended consequences in breeding programs, with hybrid bees apparently exhibiting more aggressive behavior than the parent species.</p> <p>However, the Oxitec mosquito works in exactly the opposite way – it is designed to lead to population suppression of the invasive <i>Aedes aegypti</i> mosquito, because all female mosquitoes carrying the OX5034 self-limiting gene die before reaching adulthood. Oxitec has demonstrated the efficacy of this approach in multiple trials in Brazil and elsewhere. More details of</p>	<p>https://en.wikipedia.org/wiki/Africanized_bee</p>

		Oxitec’s successful field trials are available in Webinar 4 .	
	<i>How does Oxitec compare to Wolbachia in terms of environmental impact?</i>	Oxitec’s OX5034 mosquito, and the Wolbachia-infected <i>Aedes aegypti</i> mosquito trialed in FL and elsewhere by MosquitoMate, are both intended to provide species-specific control of the invasive <i>Aedes aegypti</i> mosquito without impacting other species, either directly or indirectly.	
Questions About the Project Location, Environment and COVID			
Information about the project.	<i>What is the email address to ask questions?</i>	Email questions to florida@oxitec.com and we will endeavor to respond as quickly as possible.	
	<i>Thank you for providing this information and facilitating discussion! What is your timeline for the mosquito release?</i>	Mosquito releases are expected to start in 2021 and continue throughout the 2021 mosquito season. More information about the project, including ongoing updates, is available at https://www.keysmosquitoproject.com .	
	<i>What is in the fluorescent powder added to the boxes? I thought the fluorescent marker was inserted into the DNA of the mosquito, not dusted on top. I would like to know what it is composed of.</i>	The mosquitoes have an inbuilt fluorescent marker, the DsRed2 protein, which is produced from a gene inserted into the OX5034 mosquito DNA. This allows monitoring of OX5034 male mosquitoes and their surviving male offspring in the field. In addition, released male mosquitoes may additionally be marked using fluorescent powders. This allows Oxitec and FKMCD to distinguish between batches of males released on different days and is important for the part of the project focused on determining longevity of released male mosquitoes. This kind of procedure is routinely used for Sterile Insect Technique releases to assess longevity and dispersal of released insects.	Section G Field Protocol
	<i>How quickly to do expect to see an impact on the wild mosquito population? And</i>	In OX5034 mosquito releases in Brazil, substantial suppression (>90%) of the wild mosquito population was observed within 13 weeks of treatment.	

	<i>how would you define success for this project?</i>	This project is aimed at demonstrating the efficacy of the OX5034 mosquito in the United States. Success for this project would be the successful demonstration of the OX5034 mosquito’s efficacy, with data of sufficient quality to seek commercial approval for the OX5034 mosquito for use anywhere in the United States.	
Trial location	<i>Which habitats are wild Aedes aegypti commonly found? In which habitats will the Oxitec mosquitos be released?</i>	<i>Aedes aegypti</i> 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 <i>Aedes aegypti</i> are found and are able to transmit diseases to humans.	
	<i>Can you expand on why The Florida Keys is the first region in the US to release Oxitec mosquitoes? Is it because there is a Mosquito Control Board to liaise with? Or is there something about the region itself?</i>	<p>The Florida Keys is one of the regions in the US that has the highest <i>Aedes aegypti</i> pest pressure, due to its climate and location. The region has also experienced several dengue outbreaks in recent decades. This, together with the development of resistance to some insecticides traditionally used to control this mosquito, has led FKMCD to seek new technologies to help protect Florida Keys residents from the diseases transmitted by <i>Aedes aegypti</i>.</p> <p>The FKMCD-Oxitec project has undergone careful review from federal and state agencies prior to approval.</p> <p>In the same way as it assesses other mosquito control technologies, including <i>Wolbachia</i>, 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>	
Questions about Oxitec Mosquitoes and Conventional Mosquito Control			
Resistance, chemical applications	<i>“Organophosphate chemical spraying will continue during this trial. Please explain how this resolves the</i>	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.	

	<p><i>organophosphate resistance issue. Why not stop spraying during the trial?"</i></p>	<p>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 will apply to any treatment, including organophosphate spraying, if used.</p> <p>This is the same as for prior Oxitec projects in other countries and would typically be the same for FKMCD projects with other technologies.</p>	
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