Response to Comments to the Notice of Receipt of an Application for an Experimental Use Permit Number 93167-EUP-E

Docket EPA-HQ-OPP-2019-0274

Table of Contents

I. Introduction

In a Federal Register Notice[1] published on September 11, 2019, the Environmental Protection Agency (EPA) announced the receipt of an application for an Experimental Use Permit (EUP) to evaluate the efficacy of releasing genetically engineered (GE) mosquitoes expressing tetracycline Trans-Activator Variant (tTAV-OX5034) protein (identified by number 93167-EUP-E) as a tool for suppression of wild *Aedes aegypti* mosquito populations. In the Notice EPA made a finding, pursuant to 40 CFR 172.11(a), that this proposed EUP is of "regional or national significance" and invited public comment.

The company submitting the request, Oxitec Ltd., described the proposed product and EUP testing in the document "Description of OX5034 *Aedes aegypti* mosquito, including Active and Inert Ingredients" Document ID No. EPA-HQ-OPP-2019-0274-0002 (0002)[2] available at regulations.gov as follows:

> "The active ingredient of the OX5034 *Aedes aegypti* mosquito ("OX5034" or "OX5034 *Aedes aegypti")* is a tetracycline-repressible transactivator protein variant (tTAV - OX5034) and the genetic material necessary to produce the protein *in vivo* in female offspring of OX5034 *Aedes aegypti* matings. Female progeny inheriting the OX5034 rDNA construct express the tTAVOX5034 protein as larvae and, in the absence of tetracycline or its analogues, die in L2/L3 larval instar stages, while males survive to fully functional adulthood. This means that released OX5034 *Aedes aegypti,* reared in the absence of tetracycline, will be males that cannot bite humans or other animals, and do not transmit disease.
>
> "The inert ingredient in OX5034 *Aedes aegypti* is a fluorescent marker, DsRed2-OX5034, which aids in the detection of *Aedes aegypti* carrying the #OX5034 rDNA construct. The DsRed2 protein belongs to a family of red fluorescent proteins, which are members of a group of fluorescent proteins identified in several *Anthozoa* species. DsRed2 is a synthetically modified variant of the original red fluorescent protein isolated from a coral-like anemone, *Discosoma* spp."

In comments submitted to docket EPA-HQ-OPP-2019-0274, N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341), stated that:

[1] 84 FR 47947 (September 11, 2019)

[2] This citation to "0002" refers to Document ID No. EPA-HQ-OPP-2019-0274-0002 (i.e., the last four digits of the Document ID Number) in Docket No. EPA-HQ-OPP-2019-0274, *available at* regulations.gov. Citations to documents in the regulations.gov docket will hereinafter use the same citation form.

> "Oxitec's 1st generation self-limiting mosquito technology (OX513A) . . . , has been succeeded by the new 2nd generation self-limiting mosquito, OX5034. The OX5034 mosquito carries many of the key features of OX513A. . . ." (N. Rose 0341 p. 1)

Oxitec had applied to EPA for an EUP for its first generation technology, OX513A, but that application is no longer active (EPA File Symbol 93167-EUP-R; docket ID EPA-HQ-OPP-2017-0756). A number of commenters responding to the September 11, 2019 FR announcement refer to specific characteristics of OX513A that are not relevant to OX5034, but EPA has nonetheless responded, where appropriate, in this Response to Comments document to comments involving OX513A. Additional details on OX513A and that EUP application can be found in docket ID EPA-HQ-OPP-2017-0756.

OX5034 is described by Oxitec, Ltd., as a species-specific female-lethal trait that results in emergence of all-male progeny in the absence of tetracycline in the larval diet. The pesticidal effect of OX5034 is species-specific as it only affects the reproductive success of *Ae. aegypti* through mating between OX5034 *Ae. aegypti* males and *Ae. aegypti* females that are already present in the release area. OX5034 homozygous males alone will be released into the environment. Only female offspring from OX5034 matings are killed by the femal-lethal trait, while OX5034 hemizygous males survive to pass on the OX5034 female-lethal trait to future generations. Unlike female mosquitoes, male mosquitoes do not bite humans. With continued field releases of OX5034 homozygous males, the *Ae. aegypti* population in the treatment area is thought to progressively decline due to the reduced number of females emerging each consecutive generation.

Under the EUP, Oxitec is planning to test the efficacy of the OX5034 product by deploying OX5034 mosquito eggs and adult males in the treatment areas. For egg releases, a known quantity of OX5034 eggs will be released in mosquito rearing boxes. In the case of adult OX5034 male releases, known quantities of adult males will be released from containers either from a vehicle or on foot.

II. Overview of Comments Received on the Notice of Receipt

This document summarizes comments that EPA received in response to the September 11, 2019 Federal Register Notice. Thirty-one thousand, two hundred thirty-five comments were received in response to the Federal Register Notice. Three hundred forty-eight comments were posted for public view at https://www.regulations.gov/. One of the posted comments carried 25,577 signatures (submitted by Friends of the Earth 0345). In addition, another 5,310 comments were received as part of a mass mail campaign but were not made available in public view at https://www.regulations.gov/; these comments were identical to comment 0329, that is posted in public view. In this Response to Comments document EPA treats comment 0329 as representative of the 5,310 comments received but not posted in the public view.

Comments were received from industry, academia, professional and trade associations, state regulatory authorities, public interest groups and private citizens.

Most of the comments, including the comment with 25,577 signatures, urged that the Agency not permit testing at all or delay testing until more information on OX5034 was available. Other comments, however, supported issuance of the EUP and testing of OX5034.

An index relating the four-digit number associated with a comment to the entity making the comment can be found in the Appendix to this document. Comments quoted in this Response to Comment document were chosen to illustrate points made in comments relevant to issues directly related to determining whether to permit testing of OX5034. Many comments received simply express an opinion without providing sufficient information to allow the Agency to formulate a response. Most comments in this category, while included in the index, are not quoted in this document. Where pertinent, commenters are quoted to illustrate a point that EPA addresses in the Response to Comment document. Two comments (0289, 0291) that address issues unrelated to the OX5034 EUP application appear to have been directed mistakenly to the OX5034 docket. All comments received have been tabulated in the index.

EPA thanks all commenters for their participation in the public process.

A. Comments Supporting Issuance of the EUP and Testing of OX5034

Fifty-six comments supporting issuance of an EUP and testing of OX5034 were received. (0004, 0006, 0007, 0010, 0017, 0018, 0019, 0020, 0021, 0022, 0024, 0029, 0032, 0034, 0068, 0075, 0087, 0090, 0102, 0122, 0150, 0153, 0163, 0174, 0177, 0188, 0190, 0191, 0202, 0207, 0211, 0212, 0216, 0220, 0230, 0238, 0244, 0251, 0263, 0276, 0297, 0298, 0299, 0301, 0313, 0321, 0322, 0324, 0330, 0336, 0337, 0338, 0339, 0340, 0341, 0343). Responses to these comments can be found in Unit III of this Response to Comment document.

B. Comments Expressing Opposition to Granting the EUP

In toto, 31,174 commenters expressed opposition. Two hundred eighty-seven posted comments urged the EPA to reject the application or delay testing until more information on OX5034 is available. (0003, 0005, 0008, 0009, 0011, 0012, 0013, 0014, 0015, 0016, 0023, 0025, 0026, 0027, 0028, 0030, 0031, 0033, 0035, 0036, 0037, 0038, 0039, 0040, 0041, 0042, 0043, 0044, 0045, 0046, 0047, 0048, 0049, 0050, 0051, 0052, 0053, 0054, 0055, 0056, 0057, 0058, 0059, 0060, 0061, 0062, 0063, 0064, 0065, 0066, 0067, 0069, 0070, 0071, 0072, 0073, 0074, 0076, 0077, 0078, 0079, 0080, 0081, 0082, 0083, 0084, 0085, 0086, 0088, 0089, 0091, 0092, 0093, 0094, 0095, 0096, 0097, 0098, 0099, 0100, 0101, 0103, 0104, 0105, 0106, 0107, 0108, 0109, 0110, 0111, 0112, 0113, 0114, 0115, 0116, 0117, 0118, 0119, 0120, 0121, 0123, 0124, 0125, 0126, 0127, 0128, 0130, 0131, 0132, 0133, 0134, 0135, 0136, 0137, 0138, 0139, 0140, 0141, 0142, 0143, 0144, 0145, 0146, 0147, 0148, 0149, 0151, 0152, 0154, 0155, 0156, 0157,

0158, 0159, 0160, 0161, 0162, 0164, 0165, 0166, 0167, 0168, 0169, 0170, 0171, 0172, 0173, 0175, 0176, 0178, 0179, 0180, 0181, 0182, 0183, 0184, 0185, 0186, 0187, 0189, 0192, 0193, 0194, 0195, 0196, 0197, 0198, 0199, 0200, 0201, 0203, 0204, 0205, 0206, 0208, 0209, 0210, 0213, 0214, 0215, 0217, 0218, 0219, 0221, 0222, 0223, 0224, 0225, 0226, 0227, 0228, 0229, 0231, 0232, 0233, 0234, 0235, 0236, 0237, 0239, 0240, 0241, 0242, 0243, 0245, 0246, 0247, 0248, 0249, 0250, 0252, 0253, 0254, 0255, 0256, 0257, 0258, 0259, 0260, 0261, 0262, 0264, 0265, 0266, 0267, 0268, 0269, 0270, 0271, 0272, 0273, 0274, 0275, 0277, 0278, 0279, 0280, 0281, 0282, 0283, 0284, 0285, 0286, 0287, 0288, 0290, 0292, 0293, 0294, 0295, 0296, 0300, 0302, 0303, 0304, 0305, 0307, 0308, 0309, 0310, 0311, 0312, 0314, 0315, 0316, 0317, 0318, 0319, 0320, 0323, 0325, 0326, 0327, 0328, 0329, 0331, 0332, 0333, 0334, 0335, 0342, 0344, 0346, 0347, 0348, 0349, 0350). The 5,310 comments identical to comment 0329 but not made available in public view at https://www.regulations.gov/, as well as the 25,577 signatures submitted by Friends of the Earth (0345), are included in the total of 31,174 commenters expressing opposition.

Comments offered in opposition covered a range of topics. Examples of the comments in these categories and EPA responses to the comments can be found in Units IV through XII of this Response to Comments document.

Some of these comments were generic in nature, offering no substantive explanation for opposition. Others, however, provided technical, legal or other arguments for opposing issuing an EUP for field testing OX5034, including descriptions of scientific considerations the commenters argued need to be evaluated and considered prior to EPA determining whether to issue a permit for OX5034 testing. A small group of commenters offered opinions on what the Agency needs to do to ensure safety and the validity of the testing under the EUP.

III. Comments Supporting Issuance of an EUP for OX5034

Comments supporting issuance of an EUP to permit testing of OX5034 generally revolved around arguments explaining: (1) why these commenters believe this technology is needed; (2) why and how this technology might work; and (3) why these commenters expect the technology to be safe. Examples of the comments in these categories and EPA responses can be found below in this Response to Comment document (Unit III).

A. Comments Explaining Why Commenters Believe This Technology is Needed

Several commenters offered explanations of why they believe this technology is needed. (0006, 0032, 0068, 0102, 0122, 0150, 0163, 0207, 0238, 0263, 0299, 0301, 0339, 0343). Most of these comments focused on the risk mosquitoes present to public health, but some commenters addressed the negative consequences on the environment of using broad range chemical pesticides to control mosquitoes.

Commenter J. Morris, International Center for Law & Economics, (0343), noting that the CDC estimates that the range of *Ae. aegypti* extends throughout most of the Southern and Eastern U.S., stated that:

> "*Aedes aegypti* mosquitoes are the leading cause of several deadly diseases, including dengue, yellow fever, chikungunya and Zika. Mosquitoes are one of the deadliest animals in the world. By spreading and infecting humans with diseases such as malaria and dengue fever, they cause millions of deaths every year. . . ." (J. Morris 0343 p. 2)

Commenter A. Shelton (0207) stated that:

> "*Aedes aegypti* distribution has increased worldwide in the past two to three decades, and it is now considered to be among the most widespread mosquito species. It is widely distributed in the southern US and is a known vector of important viruses including yellow fever virus, dengue virus, chikungunya virus and Zika virus. Control of these diseases is becoming increasingly challenging because of global warming and insecticide resistance. Novel environmentally friendly, effective strategies are needed and use of GE insects can meet that challenge." (A. Shelton 0207 p. 1)

Commenter D. Strickland (0301) stated that:

> "The threat of disease transmitted by this species is severe. Dengue and chikungunya viruses continue to circulate in the Caribbean and Mexico, constituting a continuous threat to the southern United States where *Aedes aegypti* occurs. In addition, we do not know when Zika virus may reappear and its widespread transmission in even a portion of the US would be highly disruptive due to the measures that would be needed to avoid an increase in birth defects." (D. Strickland 0301 p. 1)

Commenter D. Mader (0238) stated that:

> "As a veterinarian I have concerns about the incidence of Heartworm disease in dogs, cats and ferrets. The Aedes mosquito is one of the vectors of this serious, often deadly disease. We see far more cases of animal morbidity/mortality in Monroe County than we do human illness. Anything that can be done, especially something as simple and safe at [sic] the Oxitech [sic] mosquito program, gets my full support." (D. Mader 0238 p. 1)

Commenter J. Morris, International Center for Law & Economics, (0343) stated that:

> "Given current inefficient mosquito-control methods, the high burden of vector-borne diseases, and the positive results of previous field trials of the OX513A *Aedes aegypti*

> mosquito, the International Center for Law and Economics urge the EPA to move forward as quickly as possible in approving Oxitec's request for an EUP to undertake field trials with OX5034 in Florida and Texas. Unfounded fears about this safe technology should not prevent it being implemented as part of the solution to a serious health problem." (J. Morris, International Center for Law & Economics, 0343 p. 8)

Commenter J. Morris, International Center for Law & Economics, (0343) supported his position by noting that the "burden associated with diseases transmitted by *Aedes aegypti* is very high" and while "an effective vaccine is now available for yellow fever, there are neither vaccines nor effective anti-viral medicines for several of other diseases spread by *Aedes aegypti*, namely dengue, chikungunya and Zika. As a result, the primary means of preventing these diseases is through mosquito control." He stated that:

> "Existing mosquito control techniques, including spraying with insecticides and attempting to manage breeding sites (e.g. using larvicides), are costly and often inefficient. For example, the Florida Keys Mosquito Control District spends about $1.1 million per year in the Key West area to achieve an estimated 50% reduction of the *Aedes aegypti* population. Moreover, the repeated use of chemical insecticides is leading to rising resistance worldwide, creating challenges for mosquito control pro-grams [sic]." (J. Morris, International Center for Law & Economics, 0343 p. 5) [Footnotes omitted]

Commenter S. Appemane, Vice President of Mahyco Grow Group (0150) argued that in light of the serious human health threat presented by the disease vector, *Ae. aegypti*, the EPA should "use a fair, evidence-based approach evaluating this technology and to provide Oxitec an opportunity to demonstrate the potential of its technology in the coming 2020 mosquito season." S. Appemane supported this position by stating that:

> "As no specific treatment is available for these diseases one of the best possible ways to control is to bring the vector population down to a threshold level where it is unable to spread the diseases. Conventional methods like fogging with pesticides, sanitation etc. have not been effective to that extent largely because of the breeding behaviour of the Aedes aegypti mosquitoes which usually breeds in small clean water bodies usually found in household waste containers. Oxitec's mosquito technology is a unique tool to bring the vector population down. It is a very promising tool to fight the dengue and other diseases related to Aedes aegypti as repeated releases brings down the vector population." (S. Appemane, Vice President of Mahyco Grow Group 0150 p. 2)

Anonymous (0299) argued that we need to see if strategies such as release of OX5034 males can work for the following reasons:

> " . . every flight from Miami to Boston could also introduce these invasive creatures to my location as well--so this is not just an issue of one location ultimately. . . . Climate change also puts us at higher risk in places that didn't have the same mosquito issues as before. We are already seeing increases in EEE, West Nile, and other viral threats." (Anonymous 0299 p. 2)

Anonymous (0339) noting that the "Zika virus is a continued public health threat in the USA, and the tools available to fight it are extremely limited, reminded that:

> "The pandemic is illustrative of the universal failure of vector-control programs in regions where rapid urbanization and interconnectivity promote epidemic spread. However, new vector-control approaches, such as those that involve genetically modified mosquitoes, wolbachia-transfected mosquitoes, and pyriproxyfen-based larvicide, are under evaluation.... the large numbers of susceptible persons residing in aedes-infested regions make a reemergence of ZIKV likely. Thus, there is a critical need to mobilize support and improve capacity in low- and middle-income countries to respond to future ZIKV epidemics and the next emerging pathogens." (Anonymous 0339 p. 1)

Commenter P. L. Goodman, Commissioner District II and Chairman of the Board Florida Keys Mosquito Control District (0068) stated support for issuance of the EUP noting that a new technology is needed to help in control of the *Ae. aegypti* mosquito:

> "Using all the tools available to us today, we currently control approximately 80% to 90% of the 44 types of mosquitoes that do not carry diseases but are a nuisance to the comfort to our residents and tourist. However, these tools are mostly ineffective in controlling the Aedes aegypti mosquito due to the chemical resistance it has developed and the unusual habitat in which this mosquito lives and thrives. We have worked with our suppliers and others to develop modified chemical products to focus on this mosquito and we have made limited progress." (P.L. Goodman 0068 p. 1)

Commenter P. L. Goodman, Commissioner District II and Chairman of the Board Florida Keys Mosquito Control District (0068) added that:

> "As a result of the near Herculean efforts we now employ to control the Aedes aegypti mosquito, at best we are controlling only 30 to 50% of this vector. This is much better than most Districts but is far from the 80% to 90% control experts say we need to nearly eliminate the chance of widespread infection like we experienced nine years ago with Dengue Fever. I believe our current margin of control, though still very low, helped us avoid local transmission of the Zika virus several years ago when our large neighbor to the north, Miami-Dade, experienced their problems controlling their Zika outbreaks.

> This past success is a good start for us but there remains much to do." (P.L. Goodman 0068 p. 1)

Commenter J. M. Conlon, American Mosquito Control Association, (0263) expressed full support for the granting of an EUP for OX5034, stating that:

> "Our inventory of public health pesticides available to control disease-transmitting peridomestic species is under continual regulatory challenge, which threatens to leave us defenseless in controlling adult host seeking mosquitoes if alternatives are not available. As a result, the dwindling array of control options needs augmentation if control of vector-borne diseases is to be realized." (J.M. Conlon 0236 p. 1)

Anonymous (0163) writing for IVCC, a Product Development Partnership of industry, academia and other public health stakeholders dedicated to facilitating the development, delivery and impact of novel and improved vector control solutions, stated that:

> "There are few novel vector control tools available to address global heath security. Oxitec mosquitoes are demonstrating that they can be an important tool in the fight against vector borne diseases, particularly those mosquitoes carried by the Aedes mosquito such as dengue and Zika. . . . With so few vector control tools available to tackle vector borne diseases, we have to support technologies such as Oxitec to make sure we can eliminate the diseases that these mosquitoes carry. We would support the request for EPA to expeditiously approve EUPs for technologies like Oxitecs [sic] so new tools can be evaluated." (Anonymous 0163 p. 1)

Commenter P. L. Goodman, Commissioner District II and Chairman of the Board Florida Keys Mosquito Control District (0068), noting that currently "the use of various pesticides are the main tools we currently have to assist us in our goal to reduce this mosquito" stated that:

> "The Florida Keys, a well known environmentally sensitive area, surrounded by a National Marine Sanctuary presents many challenges when spraying chemicals and these restrictions are expected to continue to expand. It is obvious to all that we will not be able to spray our way out of the growing threat this mosquito poses to us and other communities." (P. L. Goodman 0068 p. 1-2)

Anonymous (0032) stated that:

> "Please do this! Controlling invasive Aedes aegypti with so called gene drives instead of chemical pesticides will help prevent human disease while protecting honeybees and native insects from overkill. It will also reduce the public's exposure to potentially dangerous chemicals. If the haters in Texas and Florida don't want your GM mosquitoes,

please send them to my backyard near Tucson, AZ. I'd be happy to welcome them." (Anonymous 0032 p. 1)

Commenter K.S. Wasserman (0006) stated that:

> "How can something that kills only mosquitoes be bad compared to chemicals that kill everything. People say it will disrupt the food chain so how does killing everything along with mosquitoes help the food chain. Where i live you almost never see a firefly, dragonfly's, moths or butterfly's,bees [sic] or wasps. True we have very few mosquito's but we have very few anything anymore. I had 3 huge Monarch caterpillars in my garden. The chopper went over and they were toast, Not to mention what those chemicals do to people. When a chopper fly's over your car you can see the tiny brown specks all over your wind shield." (K.S. Wasserman 0006 p. 1)

B. Comments on Why and How This Technology Might Work

Several commenters offered explanations on why and how this technology might work. (0024, 0068, 0102, 0191, 0263, 0324). Some commenters pointed specifically to experience with SIT (Sterile Insect Technique) technology, including the species specificity of SIT technologies in general; others pointed out that if successful, the technology represented by OX5034 would be a welcome addition to existing control methods, and may prove easier to implement than technologies available today.

Commenter T. Nolan (0024), voicing support for OX5034, stated that:

> "The technology comprises several steps that make it unique and potentially game changing in that: 1- it comprises a species-specific way of local suppression of a pest 2- it includes a sexing technology that is genetics-based, allowing reliable production and release of a male-only population of mosquitoes; 3- the technology is designed to persist for a matter of time before gradually disappearing after releases cease, allowing an increased penetrance of the wild population of mosquitoes and greater duration of suppression." (T. Nolan 0024 p. 1)

Commenter M. Coldiron (0191) stated that:

> "Oxitec's technology has the potential to provide U.S. communities with a new, effective, and environmentally sustainable *Aedes aegypti* control solution at a time when other vector management tools face challenges." (M. Coldiron 0191 p. 1)

N. C. Leppla, representing the University of Florida Integrated Pest Management Program, (0324) cited his years of experience in conducting research and action programs utilizing SIT technology, and stated that:

> "Areawide mosquito control using SIT has not become a standard practice only because it is much more expensive and complicated than applying insecticides. Unfortunately, the cost comparisons between SIT and insecticide applications do not include the destructive impacts of the insecticides on non-target organisms. If the proposed tests of *Aedes aegypti* OX5034 are successful, this technology has the potential to provide bio-based mosquito management and substantially reduce human exposure to several devastating diseases. Moreover, the technology might be adapted for other dipteran species that vector pathogens of humans and animals." (N. C. Leppla, University of Florida Integrated Pest Management Program, 0324 p. 2)

Commenter J. M. Conlon, American Mosquito Control Association, (0263) further argued that:

> "The tTAV-OX5034 *Aedes aegypti* mosquitoes represent a most welcome and innovative technology that could serve as a valuable adjunct to existing integrated mosquito control programs if successfully tested in the United States. Prior successful usage in Brazil and the Cayman Islands speaks well to its value as a potential control agent here in the United States as well. Its potential as an added mosquito population suppressive measure to control modalities already in use cannot be underestimated. If proven to be effective, its availability would enhance (but not necessarily supplant) our current capabilities at a time when CDC has documented a three-fold increase in vector-borne diseases in the past 12 years." (J.M. Conlon 0236 p. 1)

Commenter P. L. Goodman, Commissioner District II and Chairman of the Board Florida Keys Mosquito Control District (0068) stated that:

> "At this time, I believe the use of a sterile insect technique (SIT) offers the best future solution for us . . . . This District has been involved with the SIT technology since Oxitec introduced their first generation to us over ten years ago and we are very informed about SIT technology. We are a highly technical district and have the ability to carry out a proper evaluation. Also, the fact that we are a chain of small islands increases our ability to potentially control or even eliminate this mosquito as opposed to areas with large land masses." (P.L. Goodman 0068 p. 2)

Commenter S. Black (0102) stating support for issuance of the EUP, stated that:

> "The only down side I see is selection pressure will be high suggesting that the improvement may not be long term." (S. Black 0102 p. 1)

C. Comments on Why These Commenters Believe the Technology Should Be Safe

Several commenters offered explanations of why they believe this technology should be safe. (0150, 0174, 0191, 0207, 0212, 0244, 0301, 0324, 0337). Some of the commenters argued that Oxitec's technology has been thoroughly and independently evaluated for more than 10 years. During that time products of the technology have received regulatory approvals, technical endorsements and recommendations from a range of countries and international bodies. One commenter noted that the technology has been used successfully to suppress Diamondback Moth, *Plutella xylostella*, populations. Another commenter argued that elimination of *Aedes aegypti* should not be disruptive of the test area ecology as *Aedes aegypti* is an invasive species, and although it has been present in the US for hundreds of years, it is not a part of the natural ecology of the North American continent.

Commenter M. Coldiron (0191) stated that:

> "Oxitec's technology has been thoroughly and independently studied for more than 10 years and has been the subject of more than 100 scientific studies and peer-reviewed publications. It has received regulatory approvals, technical endorsements, and recommendations from a range of countries and international bodies. In addition, Oxitec has been through more than 10 years of effort working through the U.S. regulatory system, which resulted in the U.S. FDA's Finding of No Significant Impact for its **lst** Generation technology." (M. Coldiron 0191 p. 1)[Emphasis in the original]

Commenter S. Appemane, Vice President of Mahyco Grow Group (0150) stated that:

> "Oxitecs technology has been thoroughly and independently studied for more than 10 years and has been the subject of more than 100 scientific studies and peer-reviewed publications. It has received regulatory approvals, technical endorsements, and recommendations from a range of countries and international bodies." (S. Appemane, Vice President of Mahyco Grow Group 0150 p. 2)

Anonymous (0244) stated that as a Professor of Biology, he endorsed the testing of OX5034 in the Florida Keys, stating that:

> "While many entertaining hypothetical questions have been raised by some members of the public, to my knowledge, not a single objection to the technology has been validated after rigorous scientific scrutiny and a review of decades of peer-reviewed publications supporting the proposed methodology. It is long past time that this lifesaving technology that is being used successfully worldwide should finally be tested here in the United States." (Anonymous 0244 p. 1)

Commenter A. Shelton (0207) indicating that he had worked extensively for over a decade with Oxitec on the self-limiting strain of Diamondback moth (OX4319L) *Plutella xylostella*, stated that:

> "Our greenhouse trials documented excellent suppression of P. xylostella and recovery of susceptibility to an insecticide when this strain was used (Harvey-Samuel et al. 2015 BMC Biol doi: 10.1186/s12915-015-0161-1). In 2017 we conducted the first release of a GE insect in the US using OX4319L and the trial was conducted safely. The results of these studies were promising for the use of OX4319L and were submitted to a peer-reviewed journal and are currently under review. . . . As an entomologist with extensive experience developing pest management programs, and with a deep understanding of risk assessments of various strategies, I support the concept and use of effective GE insects to control their pest populations. GE insects are species-specific so do not disrupt pollinator and other natural enemies important to an ecosystem. Furthermore, I support science-based regulations to improve pest management." (A. Shelton 0207 p. 1)

Commenter D. Strickman (0301) stated several reasons for his support, including that:

> "Aedes aegypti is an invasive species, though it has been present in the US for hundreds of years. It is not a part of the natural ecology of our continent and its elimination would not be disruptive to that ecology. Programs in the 1970s attempted to eliminate this species to stop the threat of yellow fever in the Western Hemisphere. The program succeeded in many countries, but not in the United States. The failure in the US was due to the way that Aedes aegypti infests a wide variety of container habitats in urban settings, making it difficult to implement a comprehensive control program, especially in large cities." (D. Strickman 0301 p. 1)

R.E. Goodman (0337) stated that:

> "There is no obvious risk from this transgenic mosquito. And the population is like self-limiting. On the other hand, there is great risk to humans who are bitten by wild type mosquitoes of this species, of having disease viruses transferred to them, and of having illness or passing the consequences on to their young if they are pregnant. So what is the choice? Reducing a population of mosquitoes that was much lower a few years ago (*Aedes aegypti*i) but has grown markedly with warmer weather? Or not reducing them, and letting people become infected?" (R.E. Goodman 0337 p. 1)

N. C. Leppla, representing the University of Florida Integrated Pest Management Program, (0324) referring back to his years of experience and the FDA Finding of No Significant Impact conclusion reached by the FDA evaluation of OX513A stated that:

> "I have complete confidence that there is no risk in conducting the proposed tests of *Aedes aegypti* OX5034 and there likely will be extraordinary benefits if this technology is adopted widely. Consequently, I encourage the Environmental Protection Agency to expeditiously grant the EUP for the test." (N. C. Leppla, University of Florida Integrated Pest Management Program, 0324 p. 2)

Anonymous (0212) stating support for issuing the EUP, stated that:

> "Oxitec's technology is preferable to any other system of biologic control I have encountered - it has neither the risk of parthenogenesis that Wolbachia might induce, nor the cross species gene transfer that gene drive might induce." (Anonymous 0212 p. 1)

Commenter D.S. Wilde (0174) stated that:

> "I think more study is needed, which is what the EPA is up to. Stay the course!" (Anonymous 0174 p. 1)

EPA Response to Unit III. – Comments Supporting Issuance of the EUP and Testing of OX5034. EPA recognizes the public health threat presented by the *Ae. aegypti* mosquito, an invasive species not native to the United States. *Aedes aegypti* mosquitoes vector several deadly viral diseases, including dengue that infects millions of people and likely kills approximately 20,000 humans globally per year, chikungunya, yellow fever and Zika. *Aedes aegypti* is widespread in the southern US, and as the climate warms *Ae. aegypti* is likely to extend that range. Diseases caused by viral pathogens such as dengue and chikungunya viruses continue to circulate in the Caribbean and Mexico, constituting a continuous threat to the southern United States where *Ae. aegypti* occurs. In addition, Zika virus, known to be vectored by *Ae. aegypti*, may reappear at any time. Resistance to insecticides is increasing in *Ae. aegypti* mosquito populations, including to sprayed insecticides and the larvicides used to manage breeding sites, making control efforts ever more difficult. Commenters noted that currently at best mosquito control districts are controlling only 30 to 50% of *Ae. aegypti* mosquitoes in their districts. In addition to the health threat *Ae. aegypti* presents to humans, these mosquitoes vector pathogens and parasites such a *Dirofilaria immitis* that are health threats to animals such as dogs.

EPA has taken these comments into consideration in its deliberations on whether to permit limited testing of OX5034 under FIFRA section 5 EUP provisions. EPA has also taken into consideration that an EUP represents a limited use for a limited period of time specifically for gathering data on the product to support an application for registration, while ensuring sufficient regulatory controls are in place to prevent unreasonable adverse effects on health and the environment.

With regard to the comment that OX5034 would be under high selection pressure with the implication that OX5034 may eventually lose the ability to suppress a local wild *Ae. aegypti* mosquito population, the commenter did not provide sufficient information in the context of this EUP request to enable EPA to respond to the comment. However, EPA notes that monitoring is part of the testing protocol, as are mitigation plans. Monitoring would follow the performance of OX5034 in the field while mitigation would simply be an extension of abatement control programs for both treated and untreated areas that would be in place during the EUP testing. Should EPA receive a request for registration of OX5034 under FIFRA section 3, EPA has the option at that time to consider whether a program designed to manage the potential for resistance to emerge in OX5034 is feasible and warranted.

With regard to the comment that technology such as OX5034 might be preferable to using Wolbachia in mosquitoes for population suppression purposes, as Wolbachia use might be associated with risks presented by parthenogenesis and cross-species gene transfer, the commenter did not provide sufficient information in the context of an EUP request to enable EPA to respond to the comment.

IV. Comments on Characteristics of OX5034

Several commenters voiced opinions on OX5034 characteristics. (0018, 0038, 0169, 0308, 0335, 0341). Some of these commenters were of the opinion that OX5034 contains a gene drive, while other commenters refuted this. One commenter argued that OX5034 is a new type of mosquito. Another commenter stated that the primary substantive difference between OX513A and OX5034 is the female killing mechanism genetically engineered into OX5034. One commenter refuted any suggestion that OX5034 contains a gene drive (0341).

GeneWatch UK (0335) stated that:

> "Very limited information regarding the newer OX5034 strain has been provided by the applicant in a published letter to the EPA. The main substantive difference, compared to the earlier OX513A strain, is that the genetically engineered killing mechanism in OX5034 is intended to kill the female GE mosquitoes only, with GE males surviving for multiple generations. Although there are some important differences between the OX513A strain and the 2nd generation OX5034 strain, many of the issues raised regarding the 1st generation releases remain of concern and have not been addressed." (GeneWatch UK 0335 p. 1) [Footnote omitted]

Commenter A. Purkis (0308) stated that:

> "Despite claims to the opposite, a new type of mosquito a GMO mosquito will be added to the environment under the GMO plan." (A. Purkis 0308 p. 1)

Anonymous (0169) stated that:

> "The Oxitec mosquitoes were developed using a controversial form of gene-editing known as gene drive. Gene Drive combined with CRISPR gene-editing, aims to force a genetic modification to spread through an entire population in just a few generations." (Anonymous 0169 p. 1)

Commenter B. Wray (0038) stated that:

> "Please advise if there is actual scientific data available on the OX5034. This is a gene-drive species and much greater risk of evolutionary consequences are associated with any release." (B. Wray 0038 p. 2)

On the other hand, commenter Q. Perkins (0018) stated that:

> "There are roughly 3,500 species of mosquitos in the world. About 175 of those live in Florida and we are talking about eliminating ONE of those species that carries 90% of the 390 million dengue infections that occur around the world? Um, how can this even be up for discussion? YES YES YES. Please let the Version 2 mosquitos do their job!!" (Q. Perkins 0018 p. 1)

Addressing the comments that referred to gene drives, N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) disputed any suggestion that OX5034 might be a gene drive:

> "Oxitec's self-limiting technology works in the opposite way from gene drive. Oxitec's 2nd Generation mosquitoes carry two copies of the self-limiting gene. When Oxitec's males mate with wild females, the self-limiting gene persists only in males. Females that inherit the gene cannot survive to reproduce. Therefore, the self-limiting gene gradually declines in the population gene pool and cannot persist, enabling potential population suppression across multiple generations before the gene is eliminated from the environment." (N. Rose 0341 p. 7)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) explained that:

> "Gene drive is a genetic engineering technology that propagates genes throughout a population without any off-switch. As a result, the gene drive insertion in the genome will re-occur in each individual insect that inherits one copy of the modification and one copy of the wild-type gene. The gene drive gene is thereby designed to convert wild-type (unmodified) counterparts into gene drive too. Therefore, these systems are designed to eventually become established or fixed in the population. Gene drive thus spreads and persists in the environment." (N. Rose 0341 p. 7)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) further explained that:

> "By releasing enough self-limiting male insects over a sustained period to mate with pest females and thereby reducing the number of female progeny, the pest population is suppressed. In contrast to the design of gene drive technologies, if releases of Oxitec males cease, the pest population can recover. As female carriers of the self-limiting gene cannot survive to reproduce, the self-limiting gene also cannot establish or become invasive in the wild." (N. Rose 0341 p. 7)

EPA Response to Unit IV. – Comments on Characteristics of OX5034. With regard to the comment that OX5034 carries a gene drive, EPA notes that OX5034, contrary to the assumption stated in a comment, does not contain a gene drive. A gene drive is a genetic engineering technology that propagates a particular suite of genes throughout a population by altering the probability that a specific allele will be transmitted to offspring at a higher rate than the natural 50% probability. OX5034 carries a conditional lethal self-limiting gene that prevents female offspring from surviving. Because it is self-limiting, 50% of the OX5034 offspring inheriting the self-limiting gene will be nonviable. The number of offspring having the OX5034 self-limiting gene will decline over time, until the self-limiting gene is eventually eliminated from the mosquito population. See Unit C.2, "Persistence of OX5034 transgene in the environment post-release" in the document entitled "Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient) and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 *Aedes aegypti*; Data and Information Were Provided in Support of a FIFRA Section 5 Application."[3] This document can be found in the docket established for this action (EPA-HQ-OPP-2019-0274).

With regard to the comment stating that OX5034 would be used to eliminate one of the 175 species of mosquito in Florida, OX5034 is intended to suppress *Ae. aegypti* mosquito populations; it is not intended to eliminate the species.

With regard to the comment that OX5034 is a new type of mosquito, the addition of 2 genes does not change the characterization of OX5034 as an *Ae. aegypti* mosquito. Oxitec has added two transgenes each of approximately 1×10^3 base pairs to the genome of an organism of approximately 1.38×10^9 base pairs. As noted above, these OX5034 transgenes will eventually be eliminated from the local wild *Ae. aegypti* mosquito population. Further, *Ae. aegypti* is considered a monophyletic and polyformic species, implying that the event selecting for domestication, i.e., a preference for association with and feeding on humans, happened once

[3] Hereafter referred to as the Human Health and Environmental Risk Assessment. This document can be found in the docket established for this action (EPA-HQ-OPP-2019-0274)

and all individual *Ae. aegypti* mosquitoes outside of Africa are descended from this single event. All *Ae. aegypti* phenotypes are considered to belong to the same species.

With regard to the comment that substantive differences exist between OX513A and OX5034, EPA evaluates each application for an EUP in order to determine whether the request meets the FIFRA standard for permitting the testing. This includes consideration of the quality of the data/information submitted in support of the request. The Agency carefully examined this OX5034 application prior to arriving at a decision on whether to issue the EUP.

V. Comments on Potential for Introgression of OX5034 Genes into the Local *Aedes aegypti* Mosquito Population

Several commenters expressed concern that OX5034 genes will enter the local wild *Ae. aegypti* mosquito population in the test area, and potentially cause adverse effects. (0025, 0028, 0033, 0035, 0039, 0049, 0050, 0052, 0053, 0054, 0056, 0059, 0060, 0063, 0070, 0071, 0072, 0073, 0078, 0082, 0086, 0094, 0095, 0096, 0098, 0100, 0111, 0114, 0119, 0123, 0124, 0125, 0127, 0128, 0137, 0140, 0142, 0156, 0169, 0183, 0187, 0193, 0226, 0233, 0245, 0250, 0252, 0259, 0262, 0264, 0266, 0271, 0273, 0277, 0294, 0317, 0318, 0323, 0326, 0329, 0331, 0332, 0333, 0335, 0342, 0343, 0344). These commenters raised concerns about possible risks associated with hybridization of OX5034 with the local wild *Ae. aegypti* mosquito population in the test area. The concerns revolve around possible effects associated with: (1) genes encoding the genetic characteristics of the Rockefeller/Latin mosquito strain, the strain modified to create OX5034, spreading in the native *Ae. aegypti* mosquito population, and (2) the genes expressing the active and inert ingredients, tTAV-OX5034 and DsRed2-OX5034 respectively, inserted into the Rockefeller/Latin mosquito strain to create OX5034, spreading into the local wild *Ae. aegypti* mosquito population. Commenters pointed to the recent study by Evans et al,[4] and stated that there is no guarantee that only beneficial traits would introgress into the local wild *Ae. aegypti* mosquito population.

On the other hand, some commenters attempted to rebut concerns about introgression resulting in a potential adverse effect. (0087, 0090, 0177, 0244, 0263, 0299, 0301, 0338, 0341).

[4] Evans, B. R., Kotsakiozi, P., Costa-da-Silva, A. L., Ioshino, R. S., Garziera, L., Pedrosa, M. C., Malavasi, A, Virginio, J. F., Capurro, M and Powell, J. R. (2019). Transgenic *Aedes aegypti* Mosquitoes Transfer Genes into a Natural Population. *Scientific Reports*, **9**(1), 1–6. https://doi.org/10.1038/s41598-019-49660-6

A. Will Genes from OX5034 Enter the Gene Pool of the Local *Aedes aegypti* Mosquito Population

Some commenters (0124, 0226, 0335, 0344) pointed out the likely genetic background of OX5034. For example, the Center for Food Safety (0344) stated that:

> "Oxitec's GE mosquitoes have been developed from a non-native strain (the Rockefeller laboratory strain, originally from Cuba). In the Cayman Islands, this was backcrossed into a Mexico-derived genetic background and it appears that this same strain was then used in Brazil and probably also in Panama. As described in Oxitec's draft Environmental Assessment for OX513A, originally submitted to the FDA, (pages 21 and 22), the GE strain OX513A was produced in 2002 by microinjection into individual embryos of *Aedes aegypti* from a Rockefeller strain background. The strain was made homozygous by repeated back‐crossing and then the insert was introgressed into an *Ae. aegypti* Latin strain background from Instituto Nacional de Salud Publica (INSP), Mexico. The Rockefeller strain is a common laboratory strain of *Aedes aegypti*, which appears to have been derived from a strain established in Havana, Cuba, by Carlos J. Finlay in 1881, used in the original experiments which established that *Aedes aegypti* mosquitoes are a vector for Yellow Fever." (Center for Food Safety 0344 p. 11) [Footnotes omitted]

Four commenters (0124, 0226, 0335, 0344) noted that the Oxitec's stated intent was that Rockefeller/Latin genes from OX5034 mosquito would enter the local wild mosquito population.

Anonymous (0226) explained that:

> "The idea is that mass releases of GM males will mate with wild females and their offspring will contain the femalekilling [sic] trait. This genetically engineered trait is intended to make most of the female offspring of these matings die before adulthood; however the male offspring are intended to survive and breed for multiple generations. In addition, wild female pests that have mated with the released GM males will lay eggs that inherit the GM female-killing trait . . . ." (Anonymous 0226, p. 13)

GeneWatch UK (0335) noted that:

> ". . . , because the OX5034 strain is female-killing only, GE males are expected to survive for multiple generations and this will considerably increase the spread of genes from the introduced strain into the wild population. In an online presentation, Oxitec presents this as a benefit because it argues that the released laboratory-derived strain will spread insecticide susceptibility genes into the wild mosquito population, however, there is no

> guarantee that only beneficial and no harmful traits will be spread in this way." (GeneWatch UK 0335 p. 1) [Footnote omitted]

Anonymous (0124) stated that:

> "Since the company would release millions of OX5034 males, and the transgene disappears at a rate of 50% per generation, this translates to 10+ generations of persistence without any comment regarding how to address this in the event of unintended consequences. A typical generation time for Aedes aegypti is about a month, but the persistence and hence the risk is increased by the potential for Aedes aegypti eggs to remain dormant for over a year in nature." (Anonymous 0124 p. 1)

Center for Food Safety (0344) stated that:

> "When Oxitec's GE mosquitoes breed with wild mosquitoes some of their other genetic characteristics will be passed on to the local wild mosquito population." (Center for Food Safety 0344 p. 11)

GeneWatch UK (0335) pointed out that:

> " . . , Oxitec has demonstrated the effects of rapid introgression of insecticide-susceptible traits in its own research and modelling of its GE agricultural pests." (GeneWatch UK 0335 p. 9) [Footnotes omitted]

Other commenters (0033, 0326, 0329, 0331, 0332, 0335, 0344) posited that data developed on the GE mosquito product, OX513A, that had been released into the environment during previous field testing outside of the United States, support the conclusion that OX5034 genes would introgress into the local wild *Ae. aegypti* mosquito population to form a hybrid *Ae. aegypti* mosquito population. For example, one commenter (0335) noted that with OX513A:

> "The released GE males mate and produce offspring which inherit the genetically engineered late-lethality trait. This means that most (but not all) of the GE mosquitoes' offspring die at the late larval stage, in the water where the female mosquitoes lay their eggs. GeneWatch UK has repeatedly warned . . .that this partial survival rate, even if low (a reported 3 to 4% in laboratory conditions), would lead to the establishment of hybrid mosquitoes in the environment, which might possess altered properties, including the potential for enhanced disease transmission or resistance to insecticides. A recent paper, reporting monitoring of wild mosquito populations following some of Oxitec's experiments in Brazil, has confirmed that such hybrid mosquitoes have indeed spread into the area surrounding the release sites." (GeneWatch UK 0335 p. 1)

The Florida Keys Environmental Coalition (0331) also noted the recent Evans et al study and commented that:

> "Oxitec could not see where they [sic] lab results showing that 15% of the OX513A survived 42 days, "long enough for females to take 2 blood meals and lay 2 clutches of eggs" would suggest survivability, likely without the fluorescent marker genetically inserted and hybridization with wild indigenous mosquitoes was expectable. Yet, this was shown to be true in the attached nature.com, Yale study, recently reported from the Brazil trial of the OX513A in Jacobina. . . ." (Florida Keys Environmental Coalition 0331 p. 2)

The Center for Food Safety (0344), referring to the Evans et al publication, stated that:

> "A recent paper, reporting monitoring of wild mosquito populations following some of Oxitec's experiments in Brazil, has confirmed that such hybrid mosquitoes have indeed spread into the area surrounding the release sites. Because the OX5034 strain is female-killing only, GE males are expected to survive for multiple generations and this will considerably increase the spread of genes from the introduced strain into the wild population." (Center for Food Safety 0344 p. 11)

GeneWatch UK (0335) echoed this comment stating that:

> ". . . due to the survival of GE males for multiple generations, the OX5034 strain is expected to increase, rather than reduce, the spread of genes from the released GE non-native strain into the wild *Aedes aegypti* mosquito population, compared to the OX513A strain." (GeneWatch UK 0335 p. 2)

L.M. Castro (0332) stated that:

> "A risk-benefit analysis of the proposed release of genetically modified mosquitos suggests that a significant risk of genetic material transfer exists in connection to this Experimental Permit to Combat Mosquitoes. This assertion is supported by the work of researchers from Yale University and Brazil (Evans et al.). They report that "genetic sampling from the target population six, 12, and 27–30 months after releases commenced provides clear evidence that portions of the transgenic strain genome have been incorporated into the target population" and "release of the OX513A has led to significant transfer of its genome (introgression) into the natural Jacobina population of *Aedes aegypti*. The degree of introgression is not trivial. Depending on sample and criterion used to define unambiguous introgression, from about 10% to 60% of all individuals have some OX513A genome . . . . Even if the strain of Oxitec mosquitoes released in the USA is not the same as the one released in Brazil, the point is that the

> failings of the proposed experiment will only be known after-the-fact and the impacts will most likely be irreversible." (L.M. Castro 0332 p. 1)

Quoting a note in the September 17, 2019 issue of The Scientist, L.R. Marshall (0033) added that:

> "The biotech company Oxitec began releasing hundreds of thousands of genetically engineered mosquitoes in the city of Jacobina between 2013 and 2015. The idea is that genetically modified (GM) males would mate with wildtype females and pass on a gene that kills their offspring before they themselves can breed, ultimately knocking down Jacobinas mosquito populations. The study's authors, who are not affiliated with Oxitec, began sampling mosquitoes in Jacobina before, during, and after the deployment of the GM insects. They created a genetic panel that distinguished the wildtype mosquitoes from the introduced ones and found that insects analyzed more than two years after the releases stopped were progeny of both wildtype and mutant, or OX513A, lineages. The degree of introgression is not trivial, the authors write in their report. Depending on sample and criterion used to define unambiguous introgression, from about 10% to 60% of all individuals have some OX513A genome." (L.R. Marshall 0033 p. 2)

Referring to the Evans et al paper, Anonymous (0329) argued that Oxitec's claims that "its GM mosquitoes are self-limiting and will result in a sustainable decrease in the wild population" are not reliable:

> "According to a peer reviewed Yale study, the GM mosquitoes released in Brazil reproduced and their GM genes contaminated the wild mosquito population. The Yale study also found that the release in Brazil did not result in a sustainable decrease in the mosquito population." (Anonymous 0329 p.1)

GMO Free USA (0326), requesting an extension of the comment period, echoed the importance of the recent paper by Evans et al stating that:

> "New research has been published this week on the efficacy of a release of Oxitec's genetically engineered mosquitoes on mosquito populations in Brazil. The study, published by Yale University scientists in the journal Nature, documented unexpected and unintended consequences from the release. Not only did mosquito population numbers bounce back up in the months following the test, but some of the native mosquitoes retained genes from the engineered mosquitoes. https://www.nature.com/articles/s41598-019-49660-6" (GMO Free USA 0326 p.1)

B. Comments on Whether Potential Problems Might Arise from Introgression of OX5034 Genetic Material into the Wild Local *Aedes aegypti* Mosquito Population

Several commenters argued that introgression of OX5034 genetic material into the local wild *Ae. aegypti* mosquito population could present problems that should be taken into consideration. (0095, 0135, 0137, 0199, 0226, 0263, 0302, 0318, 0329, 0332, 0335, 0342, 0344).

For example, the Center for Food Safety (0344) stated that:

> ". . , there is no guarantee that only beneficial and no harmful traits will be spread." (Center for Food Safety 0344 p. 11)

Comments arguing that introgression of OX5034 genetic material into the local wild *Ae. aegypti* mosquito population may cause problems revolved around those positing that potential consequences could result from: (1) genes of the Rockefeller/Latin Mosquito strain introgressing, or (2) from the tTAV-OX5034 and DsRed2-OX5034 genes introgressing.

Comments attempting to dispel concerns were also submitted. (0087, 0090, 0177, 0263, 0338, 0341, 0343).

1. Comments Positing Potential Consequences of Genes of the Rockefeller/Latin Mosquito Strain Used to Create OX5034 Introgressing into the Local Wild *Ae. aegypti* Population

Concerns identified with the introgression of genes of the Rockefeller/Latin Mosquito Strain used to create OX5034 into the local wild *Ae. aegypti* population primarily focused on issues of whether: (1) the hybrid population might have greater vector competency than the original local wild *Ae. aegypti* population, and (2) the hybrid population might display hybrid vigor with a greater a potential to establish and maintain itself in the environment. For example,

The Center for Food Safety (0344) argued that:

> "The use of a non-native strain risks spreading altered disease transmission properties into the wild mosquito population and/or creating strains which exhibit "hybrid vigour" (for example, becoming more fertile, as has been demonstrated for hybrid strains of other mosquito species)." (Center for Food Safety 0344 p. 11)

Quoting José Maria Gusman Ferraz, a researcher at Ecological Engineering Laboratory of Unicamp (the University of Campinas, a public research university in the state of São Paulo, Brazil), commenter L.M. Castro (0332) added that:

> "The study shows that there was a gene exchange, and that in this exchange the wild mosquitoes incorporated genes from another [transgenic] variety, resulting in hybrid insects, which usually have greater vigour and are more potent . . . . What we have now is a 'super mosquito' that can grow in environments where others might not grow." (L.M. Castro 0332 p. 1-2) [L.M. Castro (0332) noted that this was translated from the original Portuguese by GMWATCH]

The Center for Food Safety (0344) and GeneWatch UK (0335) went on to argue that:

> "Different strains of the same species are found in different places and some strains are more resistant to insecticides than others or better transmitters of disease (the four serotypes of the dengue virus and/or other viruses, such as chikungunya, zika and Yellow Fever). *Aedes aegypti* may transmit zika, chikungunya, yellow fever and four different serotypes of dengue, yet strains may vary significantly in their ability to transmit these tropical diseases. In the case of zika, little is known about vector strain variation and its consequences. The possible introduction of such traits needs to be considered very seriously. Harm to people's health can be increased if some serotypes or viruses can be transmitted more easily by the introduced strain than they were by the wild species already in the area, or if the strain is resistant to insecticides." (Center for Food Safety 0344 p. 11; GeneWatch UK 0335 p. 9) [Footnotes omitted]

Anonymous (0137) questioned:

> ". . . now there is contamination of the genetic code of wild mosquitos. We do not yet fully understand the repercussions of this change. Will these mosquitos now carry different diseases? Will they be more resistant to other methods of killing off the population?" (Anonymous 0137 p. 1)

Anonymous (0199) stated that:

> "The demonstration of introgression resulting in increased genetic variation in Jacobina aegypti is a monumental setback. Oxitecs [sic] response is classic typology that introduced Gene's have no consequences and they assert that there is no danger because it is one species. A huge literature of nearly 50 years has validated significant population genetic differentiation between aegypti populations around the world and documented population variation in vector capacity for dengue, Zika and yellow fever viruses, mosquito behavior and insecticide resistance. To assert species specific variation is all the same is naive at best." (Anonymous 0199 p. 1)

GeneWatch UK (0335) and the Center for Food Safety (0344) reminded that:

> "Biting females may transmit disease even if they are disease-free on release (or at the time of birth in the environment), since they may encounter one of the diseases for which the *Aedes aegpti* [sic] mosquito is a vector (e.g. dengue, zika, chikungunya, yellow fever) by biting an infected person or animal, and spread that disease by subsequently biting an uninfected person or animal." (GeneWatch UK 0335 p. 6; Center for Food Safety 0344 p. 8))

Regarding the possibility of "hybrid vigor," L.M. Castro (0332) stated that:

> "While the term 'super-mosquito' may sound like hyperbole, the fact is that enlarging the genetic the genetic pool [sic] of a pest known for its significant public health impacts is hardly in the best interest of American citizens and it is definitely not a good strategy to prevent pesticide resistance." (L.M. Castro 0332 p. 2)

J. Butler (0135) stated that:

> "Recently, the public has learned that Oxitec's GM mosquitoes released in Brazil bred and reproduced 'super mosquitoes 'and have become an alarming environmental hazard. These GM mosquitoes successfully reproduced and the resulting hybrid population is now spreading out of control, are more difficult to control, and, therefore, more prone to carry mosquito born [sic] diseases." (J. Butler 0135 p. 1)

J. Smith (0095) stated that:

> "Oxitec mosquito, OX513A, shows that Oxitecs [sic] claims that its GM mosquitoes are self-limiting are unreliable. The GM mosquitoes ended up breeding with native mosquitoes, transferring their genes into the natural population and forming hybrid mosquitoes that may be more vigorous and have a different disease-carrying potential. Therefore Public safety concerns must be respected and this experimental release must not be allowed to go ahead. It is also not known what impacts cross-breeding between GM mosquitoes and native mosquitoes may have on their ability to transmit diseases." (J. Smith 0095 p. 1)

GeneWatch UK (0335) and the Center for Food Safety (0344) pointed out that:

> "For comparison, in the UK, Oxitec has been prevented from releasing a GE diamondback moth (an agricultural pest) because of concerns about the use of a North American background strain, which is subject to controls under plant pest control regulations." (GeneWatch UK 0335 p. 10, Center for Food Safety 0344 p. 12) [Footnote omitted]

Quoting José Maria Gusman Ferraz, a researcher at Ecological Engineering Laboratory of Unicamp (the University of Campinas, a public research university in the state of São Paulo, Brazil), L.M. Castro (0332) noted that "there was gene exchange":

> " . . . resulting in hybrid insects, which usually have greater vigour and are more potent – yet there are no studies on these hybrids. Even less is known about the hybrid's efficiency in virus transmission, which may even be higher." (L.M. Castro 0332 p. 1-2) [L.M. Castro (0332) noted that this was translated from the original Portuguese by GMWATCH]

Commenter Anonymous (0329) stated that:

> "The long term impact of the GMO genes in the wild population has not been studied and could cause the wild population to be more virulent, resulting in higher rates of mosquito borne disease." (Anonymous 0329 p. 1)

2. Comments Raising Questions About Potential Consequences of the tTAV-OX5034 and DsRed2-OX5034 Genes Introgressing into the Local Wild *Aedes aegypti* Mosquito Population

Several commenters in this grouping raised questions concerning the potential for the genes engineered into OX5034, i.e. tTAV-OX5034 and DsRed2-O5034, to introgress into the local wild *Ae. aegypti* mosquito population.

Friends of the Earth (0342) stated that:

> "According to the new application, Oxitec's male OX5034 GE mosquitoes are 'female-killing', which means that the GE males would mate with wild female mosquitoes. The female offspring would theoretically die, and the genetically engineered male OX5034 mosquitoes would theoretically survive into adulthood. This means that the OX5034 mosquitoes would increase the spread of the genetically engineered material into wild mosquito populations over several generations. However, there is no information or data about whether the spread of these genes would be beneficial to the environment or public health." (Friends of the Earth 0342 p. 2)

Commenter J. Rubin (0318) stated that:

> "No matter how infrequent, there is evidence of horizontal gene transfers from invertebrates to mammals. Therefore, EPA must not allow this experiment in our interconnected, living environment. Has EPA calculated how quickly these introgressed gmo genes--now loose in the world-- will travel through the entire mosquito

> population? Perhaps beyond mosquitoes? And the impact of that introgression on our ecosystem? If not, what business have you allowing such a genetic manipulation?" (J. Rubin 0318 p. 2)

Anonymous (0329) stated that:

> "GM mosquitoes could more effectively transmit mosquito-borne diseases compared to Aedes adgypti [sic] already in Florida and Texas." (Anonymous 0329 p. 2)

3. Comments Attempting to Dispel Concerns About Introgression

Several commenters (0087, 0090, 0177, 0263, 0338, 0341, 0343) voiced opinions discounting concerns about introgression.

Commenter J. Morris, International Center for Law & Environment, (0343) stated that:

> "When OX5034 are released to mate with wild females, the female offspring inherit a self-limiting gene that causes them to die before becoming functional adults. Because male offspring survive, the second generation of males pass on the self-limiting gene. However, in third and subsequent generations, only half the offspring inherit the self-limiting gene; the other half are wild type. As a result, the presence of the self-limiting gene in the population naturally declines to extinction in a few generations". (J. Morris, International Center for Law & Environment, 0343 p. 6) [Footnote omitted]

Commenter J.M. Conlon, Technical Advisor to the American Mosquito Control Association, (0263) stated that:

> "Oxitec's new OX5034 self-limiting *Aedes aegypti* is designed to provide additional cost-effectiveness and potentially higher rates of vector control. In recent trials in Brazil, releases of these 2nd Generation male mosquitoes achieved up to 96% suppression of wild *Aedes aegypti* populations in the city of Indaiatuba, Brazil. This level of protection promises to achieve requisite control of vectors substantially below disease transmission threshold if employed judiciously within the United States and its territories." (J.M. Conlon 0263 p. 2)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) indicated that Oxitec Ltd., would like to address a few salient topics raised in comment and thus was providing additional necessary context, information and technical details to the docket.

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) stated that:

> "Introgression into the wild mosquito population of natural mosquito genes present in OX5034 is expected to occur, and these genes are also expected to disappear from the environment over time. The natural background genetics of OX5034 were selected to ensure that OX5034 is susceptible to commonly-used insecticides, meaning that introgression of these genes into the wild population has the potential to help make the wild population less resistant to insecticides used to control mosquitoes." (N. Rose 0341 p. 7)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) added that:

> "The natural genes passed on by the few surviving OX513A mosquitoes died out in treated areas after releases stopped;" (N. Rose 0341 p. 6)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) also stated that:

> " . . , if any effect were to occur as a result of OX513A background genetics being introgressed into the local population, the effect would be expected to be beneficial, as introgression of insecticide-susceptible alleles would be expected to occur, restoring the effectiveness of insecticides against a local population that may have developed resistance." (N. Rose 0341 p. 7)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) responding to the Evans et al statement on OX513A that "introgression may introduce other relevant genes such as for pesticide resistance" stated that:

> " . . . OX513A is susceptible to standard insecticides (for example, pyrethroids and organophosphates) used for mosquito control. . . . (Carvalho et al., 2015). . . . (Patil et al., 2018)." (N. Rose 0341 p. 6)

Also responding to the Evans et al paper, N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) pointed out that:

> ***"Oxitec has demonstrated that OX513A is not resistant to commonly used insecticides*** (Carvalho et al., 2015)**.** (N. Rose 0341 p. 3) [Emphasis in the original]

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) also stated that:

> "Natural genes carried by Oxitec mosquitoes do not confer increased capacity to transmit disease nor resistance to commonly used insecticides;" (N. Rose 0341 p. 3)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) responding to the Evans et al statement that it is not known "what impacts introgression from a transgenic strain of *Ae. aegypti* has on traits of importance to disease control and transmission" stated that there is an:

> " . . . extensive body of literature that demonstrates that many of the factors most likely to affect vector competence are not genetic, but environmental, relating to the mosquito's microbiome and immune response, and relating to the genetics of the virus rather than the vector." (Tabachnick, 2013; Palmer, Varghese & Van Rij, 2018; Souza-Neto, Powell & Bonizzoni, 2019)." (N. Rose 0341 p. 5)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) responding to the Evans et al hypothesis that introducing background genetics would lead to increased 'hybrid vigor' stated that:

> ***"The data published in this paper and in the entire body of peer-reviewed literature do not support this hypothesis***." (N. Rose 0341 p. 5) [Emphasis in the original]

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) also pointed out that:

> *"Aedes aegypti* is an invasive, non-native mosquito species in Brazil and throughout most of the world, and thus the natural background genetics of Oxitec's strain, along with the wild *Aedes aegypti* found locally in Brazil, are both introduced to the area." (N. Rose 0341 p. 3)

Commenter J. Conrow (0090) contributed a report written for the Cornell Alliance for Science blog describing media and reactions to the Evans et al paper:

> https://allianceforscience.cornell.edu/blog/2019/09/speculative-scientific-paper-drives-false-media-reports-gmo-mosquitoes/ (J. Conrow 0090 p.1)

Anonymous (0087) also contributed a media report describing media and other reaction to the Evans et al report. The commenter stated that the article:

> " . . . highlights that six of the 10 authors of the Scientific Reports article have retracted their support for the paper." (Anonymous 0087 p. 1)

Anonymous (0177) noted that despite negative headlines, "the science underlying the headlines actually supported the effectiveness and safety of the Oxitec mosquito:

> "The EPA should consider carefully the scientific data in the Evans et al, 2019 paper regarding safety and effectiveness of Oxitec's mosquitoes, and not be swayed by the sensationalist headlines about the paper." (Anonymous 0177 p. 1)

Commenter J.M. Conlon (0338), American Mosquito Control Association, stated that:

> " . . . there were no mosquitoes found carrying OX513A transgenes that kill offspring and fluorescently label the GMO mosquitoes. The new DNA found was genetic background from the cross between *Aedes aegypti* strains from Cuba and Mexico forming the production hybrid of OX513A mosquitoes being released." (J.M. Conlon 0338 p.2)

EPA Response to Comments in Part V.A. – Will Genes from OX5034 Enter the Gene Pool of the Local Wild *Aedes aegypti* Mosquito Population. EPA has carefully evaluated a recent paper examining the applicant's 1st generation product, OX513A, that describes evidence of introgression of OX513A background genes (i.e., non-transgenes) into the local wild *Ae. aegypti* mosquito population after releases in Brazil of OX513A males containing the self-limiting tTAV gene (Evans et al. 2019). Although that paper investigated OX513A mosquitoes, the findings are relevant to the evaluation of OX5034 where a higher degree of introgression is likely to be observed due to the larval survival rates of OX5034. EPA has concluded that introgression of OX5034 strain genetics into the local wild *Ae. aegypti* mosquito population is likely to occur during releases of OX5034; however, as described below, the risk resulting from such introgression is negligible.

EPA Response to Comments in Part V.B. – Comments on Whether Potential Problems Might Arise from Introgression of OX5034 Genetic Material into the Wild Local *Aedes aegypti* Mosquito Population. EPA has carefully evaluated whether any potential risk consequences could be associated with introgression of OX5034 genes into the local wild *Ae. aegypti* mosquito population of the test area. That evaluation can be found in a Memorandum entitled "Summary of the Data and Information Related to Vectorial Capacity Presented for the New Product OX5034 (EPA File Symbol: 93167-EUP-E) Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034), a Variant of the Modified *Discosoma* spp. DsRed2 Protein, and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 *Aedes aegypti*. Data and Information Were Provided in Support of a FIFRA Section 5 Application."[5] This Memorandum can be found in this docket (EPA-HQ-OPP-2019-0274). Considerations discussed in this Memorandum are responsive to many of the concerns raised. Parts where this Memorandum is responsive to concerns raised will be noted in the discussion below where relevant.

[5] Hereafter referred to as the Memorandum on Vectorial Capacity. This Memorandum can be found in this docket (EPA-HQ-OPP-2019-0274).

With regard to comments questioning whether introgression could result in greater vector competency, i.e., the ability of an *Ae. aegypti* mosquito to transmit viral disease, the Memorandum concludes that given the potentially limited role of mosquito genetics in vector competence as well as the known temporal and spatial variation of vector competence among mosquito populations, introgression of OX5034 strain genetics into the local wild *Ae. aegypti* mosquito population would not be expected to increase the vector competence of the local wild mosquito population beyond the natural variation and evolution that occurs in populations of *Ae. aegypti*.

With regard to comments questioning whether introgression could result in hybrid vigor, the Memorandum on Vectorial Capacity concludes that there is no indication that mosquitoes from wild populations are suffering from inbreeding depression that matings between OX5034 mosquitoes and local wild *Ae. aegypti* mosquitoes would result in hybrid vigor in the offspring. Data provided by the applicant combined with literature searches indicate that introgression of OX5034 strain genetics is unlikely to result in the increased fecundity or longevity of wild mosquitoes, factors that could also increase robustness of the wild mosquito population.

With regard to the comment that it is classic typology to argue that introduced genes have no consequences simply because OX5034 is part of a single species, the analysis in the Memorandum examines the question of whether there are significant differences between OX5034 and wild *Ae. aegypti* mosquito populations in important traits relevant to public health and concludes that there are not.

With regard to comments claiming that following testing of OX513A in Jacobina Brazil "the resulting hybrid population is now spreading out of control, are more difficult to control, and, therefore, more prone to carry mosquito born [sic] diseases," the commenter did not provide any information or data supporting this claim, nor have any recent publications supported the contention, e.g., the article by Evans et al.

With regard to the comments on the potential for the tTAV-OX5034 and DsRed2-OX5034 genes to introgress into the local wild *Ae. aegypti* mosquito population, these genes are self-limiting and while they are intended to be present in the local wild *Ae. aegypti* mosquito population (with only males carrying the OX5034 traits surviving to adulthood) during the trial and for some limited period post the final release of male OX5034 mosquitoes, they are expected to eventually be eliminated. Given its self-limiting behavior, the tTAV-OX5034 gene is expected to be eliminated from the local *Ae. aegypti* mosquito population within 10 generations post release of male OX5034 mosquitoes. Importantly, tTAV-OX5034 and DsRed2-OX5034 are located on the same genetic cassette and are therefore inherited together; see Unit II.A.1 of the Human Health and Environmental Risk Assessment found in the docket established for this action (EPA-HQ-OPP-2019-0274). Thus, due to the self-limiting behavior of tTAV-OX5034 gene and the genetic linkage between tTAV-OX5034 and DsRed2-OX5034, it is highly unlikely that

these genes will travel through the entire mosquito population, a suggestion offered by one commenter, let alone be transferred horizontally to other organisms.

Further discussion regarding the expectation that the transgene will be eliminated from the local *Ae. aegypti* mosquito population can be found in Unit II.C.2, "Persistence of the OX5034 transgene in the environment," of the Human Health and Environmental Risk Assessment. Additionally, further discussion regarding the low risk of transfer of the OX5034 cassette to other species outside of *Ae. aegypti* can be found in Unit II.D.2.a.ii, "Nontarget Insects," of the Human Health and Environmental Risk Assessment.

VI. Comments Questioning Whether Adult OX5034 Female Mosquitoes or Their Offspring Females Expressing OX5034's Engineered Genes Might Occur in the Test Areas

Comments offered suggested mechanisms through which biting *Ae. aegypti* females carrying tTAV-OX5034 and/or DsRed2-OX5034 genes might be present in the test area. Comments on routes through which this might occur include: (1) contamination of OX5034 adult male releases by adult OX5034 biting females or incomplete penetrance of the OX5034 trait; (2) failure of the tetracycline-dependency gene in OX5034 to have complete penetrance; (3) sufficient tetracycline in the environment to allow tetracycline-dependent females to mature to adults; and (4) emergence of resistance.

A. Comments Questioning the Penetrance of the OX5034 Trait and Positing that Contamination of Adult Male OX5034 Mosquito Releases by Adult OX5034 Females Could Occur

Some commenters (0014, 0038, 0039, 0048, 0124, 0137, 0142, 0226, 0329, 0331, 0335, 0342, 0344) expressed concern that *Ae. aegypti* females carrying OX5034 genes, i.e., mosquitoes that bite, might at some point be in the test areas. A number of the comments referred to past trials involving the OX513A mosquito.

Anonymous (0329) stated that:

> "Oxitec/Intrexon plans to potentially release billions of mosquitoes and it is unclear how many of them will be biting and disease spreading females." (Anonymous 0329 p. 1)

Friends of the Earth (0342) commented that:

> "There is also no publicly accessible information about the likelihood that the female OX5034 mosquitoes could survive into adulthood, particularly in the presence of

> tetracycline. Similar to previous applications, Oxitec's claim that no biting GE females will survive in the environment is unsubstantiated. There needs to be an EIS with quantitative data about the effectiveness of the OX5034 mosquito's engineered "female-killing" trait, and the public should not be asked to rely on Oxitec's claims." (Friends of the Earth 0342 p. 2)

GeneWatch UK (0335) stated that:

> "It is also notable that no public information has been provided in the Docket or elsewhere relating to the survival rates of GE females to adulthood, in the presence or absence of sources of tetracycline: this makes it impossible to assess Oxitec's claim that no biting GE females will be released or survive to adulthood." (GeneWatch UK 0335 p.2)

Commenter B. Wray (0038) stated that:

> "We request much greater testing and documentation of the science and performance of the OX5034. In light of the survival of the OX513A, we must have proof of what the OX5034 survival rates are for females. Prove it is safe!" (B. Wray 0038 p. 2)

Some commenters (0226, 0331, 0335, 0342, 0344) invoked past experience to support their concerns. GeneWatch UK (0335) and the Center for Food Safety (0344) stated that "Oxitec aims to release only male GE mosquitoes, however in practice large numbers of female GE mosquitoes – which may bite and transmit disease - have been released during past experiments with Oxitec's OX513A GE mosquitoes." (GeneWatch UK 0335 p. 5; Center for Food Safety 0344 p. 6)

GeneWatch UK (0335) added that while Oxitec now states that "its new OX5034 strain will avoid this problem because it provides "genetic separation to 100% males":

> " . . , Oxitec has provided no evidence that the female-killing mechanism engineered into the OX5034 strain is 100% effective. It is essential that such evidence is published and made available for independent scrutiny and consultation in order to assess the risk of release of female GE mosquitoes in the proposed experiments." (GeneWatch UK 0335 p. 5)

Pointing to the Evans et al paper, Friends of the Earth (0342) pointed out that while "Oxitec's application states that female offspring of the OX5034 mosquitoes are expected to die before adulthood and therefore people won't be exposed to biting female mosquitoes":

> "Oxitec has not provided evidence for this claim. Previously, Oxitec made claims about mosquito sterility, but a recent study from the Powell lab at Yale University confirmed that Oxitec claims that the mosquitoes were sterile were not true, and that some of the offspring of Oxitec's genetically engineered mosquitoes survived into adulthood. Genetic material was spread into wild populations of mosquitoes, and the direct and indirect environmental and health impacts of the new mosquitoes carrying novel genetics were concerning." (Friends of the Earth 0342 p. 4) [Footnote omitted]

In further support of their concerns, GeneWatch UK (0335) and Center for Food Safety (0344) pointed to past experience with biting females and Oxitec's OX513A mosquito strain:

> "Oxitec used a mechanical method to sort its OX513A GE mosquitoes by size, with the aim of releasing mainly male mosquitoes, which do not bite. In 2014, Oxitec published a number of figures on the number of biting female GE mosquitoes that are inadvertently released. In practice, these criteria were often exceeded. For example, checks by the Mosquito Research and Control Unit (MRCU) in the Cayman Islands on one production batch on May 12th, 2017 revealed 9 females in one release pot of 500 (1.8%), nine times the agreed level. The Cayman Islands' report also shows significant increases (spikes) in adult female mosquito numbers (green line in Figure 1B) in the release area 5 to 7 weeks after the releases begin, and again 7 to 8 weeks after the releases are increased. These spikes in the adult female population exceed 150% of the comparator population, but their true extent is not shown as the peaks are cut off on the graph. These female GE mosquitoes pose a risk to the public because they can bite and transmit disease. Emails released as a result of a Freedom of Information (FOI) request in the Cayman Islands highlight "*a significant increase in the number of female mosquitoes collected in the treatment area*", rather than a decrease, which is thought to be due to the accidental release of GE female mosquitoes. The emails reveal a high level of concern about the inadvertent release of GE female mosquitoes, from the Mosquito Research and Control Unit (MRCU) scientist with access to the data." (Center for Food Safety 0344 p. 7; GeneWatch UK 0335 p.5) [Emphasis in the original] [Footnotes omitted]

Anonymous (0226) expressed the opinion that:

> " . . the genetic trait is passed on to both the male and female offspring that are produced when the released GM male mosquitoes mate with wild females. Some of these GM female larvae will also survive to adulthood." (Anonymous 0226 p. 9-10)

Florida Keys Environmental Coalition (0331) stated that:

> "Quantified evaluation of any potential female hatching needs to be monitored by observing a statistically significant number of eggs reaching fruition." (Florida Keys Environmental Coalition 0331 p. 3)

Florida Keys Environmental Coalition (0331), in their resubmitted 2017 comments (0756-0426) noted that with OX513A:

> "The empirical data from the Cayman field trial, where females recovered in traps suggest that between 1% to 2.8% of the released mosquitoes are female. This is a very significant number and using the numbers in the spreadsheet I provided, the number of females released would grow to 66 million in Harris County, TX and 44 million in the Keys, if all 450 acres were covered." (Florida Keys Environmental Coalition 0331 p. 2)

Anonymous (0226) stated that:

> "Emails released as a result of a Freedom of Information (FoI) request in the Cayman Islands highlight "a significant increase in the number of female mosquitoes collected in the treatment area, rather than a decrease, which is thought to be due to the accidental release of GM female mosquitoes. The emails reveal a high level of concern about the inadvertent release of GM female mosquitoes, from the MRCU scientist with access to the data. A 2017 report includes female adult mosquito numbers collected from traps in the published data. The graph shows significant increases (spikes) in adult female mosquito numbers in the release area five to seven weeks after the releases begin, and again seven to eight weeks after the releases were stepped up." (Anonymous 0226 p. 10)

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "Oxitec's approach to reducing the reproductive capacity of its GE mosquitoes has a number of major weaknesses. Firstly, the killing trait may not be fully penetrant (meaning not all the GE insects will die) and is late-acting (meaning the insects are not sterile, but mostly die at the late larval stage). In the case of its OX513A strain, Oxitec published evidence that 3 to 4% of these GE mosquitoes unintentionally survived to adulthood: however, no information has been provided on the penetrance of the female-killing trait in OX5034. This means it is impossible to assess how many GE female mosquitoes might survive to adulthood." (GeneWatch UK 0335 p.7; Center for Food Safety 0344 p. 8-9) [Footnote omitted]

Friends of the Earth (0342) noted that:

> " . . . with the OX513A mosquitoes, 3 to 4 percent of Oxitec's mosquitoes survived into adulthood in the lab in the absence of tetracycline despite carrying the lethal gene. There is no data to confirm the survival rate of the GE females in the environment, both with or without the presence of tetracycline." (Friends of the Earth 0342 p. 3-4) [Footnote omitted]

Anonymous (0226) stated that:

> "In the laboratory, 3% of the offspring of Oxitec's GM mosquitoes survive to adulthood, even in the absence of the antibiotic tetracycline. When GM mosquitoes were fed cat food containing industrially farmed chicken, which contains the antibiotic tetracycline, the survival rate increased to 15-18%. Oxitec . . . admitted to an 18% survival rate in a published paper." (Anonymous 0226 p. 11)

B. Comments Questioning Whether Sufficient Tetracycline Occurs in the Test Environment to Allow Tetracycline-Dependent Female *Ae. aegypti* Mosquitoes to Mature to Adults

Some commenters (0221, 0335, 0342, 0344) suggested that consideration be given to the possibility that there may be sufficient tetracycline present in the testing environments to influence the ability of tetracycline-dependent female *Ae. aegypti* to mature to biting adults.

The Center for Food Safety (0344) and GeneWatch UK (0335) commented that:

> "In its 2004 report, the National Research Council's (NRC's) Committee on the Biological Confinement of Genetically Engineered Organisms (GEOs) states that biological confinement (bioconfinement) includes the use of biological barriers, such as induced sterilization, that prevent GEOs or transgenes from surviving or reproducing in the natural environment (page 15). The report emphasises the importance of considering the large scale at which bioconfined organisms could be released and the possibility that even carefully planned, integrated bioconfinement methods could fail. It concludes that research is needed to characterize potential ecological consequences of bioconfinement methods and to develop methods and protocols for assessing environmental effects should confinement fail (page 12)." (Center for Food Safety 0344 p. 8; GeneWatch UK 0335 p. 7) [Footnote omitted]

The Center for Food Safety (0344) and GeneWatch UK (0335) pointed out that:

> " . . . the lethality trait is conditional: the company uses the common antibiotic tetracycline as a chemical switch to turn off the killing mechanism, allowing the insects to be bred in the laboratory. This mechanism can therefore fail if the GE mosquitoes encounter high enough levels of tetracycline in the environment." (Center for Food Safety 0344 p. 9; GeneWatch UK 0335 p. 7)

Friends of the Earth (0342) pointed out that:

> "There is no data to confirm the survival rate of the GE females in the environment, both with or without the presence of tetracycline." (Friends of the Earth 0342 p.3-4)

Some commenters (0082, 0342, 0344) stated that tetracycline is a commonly used antibiotic and it is possible that mosquito larvae might encounter sufficiently high enough concentrations of the antibiotic to allow the larvae to mature to adults. Commenters indicated 3 routes by which sufficient concentrations of tetracycline might become available to allow *Ae. aegypti* larvae to mature to adult mosquitoes: through agricultural production use, in sewage, or in food production and animal husbandry.

1. Agricultural Production

Some comments (0082, 0342, 0344) offered suggestions on agricultural processes that might provide routes through which tetracycline could be present in the test environment.

For example, Friends of the Earth (0342) commented that:

> " . . , tetracycline is a common antibiotic used in agriculture production, and Florida citrus growers use significant amounts of tetracyclines (oxytetracycline) on agricultural lands as a pesticide in efforts to control the bacteria responsible for the Citrus Greening disease. The significant presence of tetracycline in the environment may obviate the lethal trait in the GE mosquitoes and their offspring could survive and continue to breed." (Friends of the Earth 0342 p.3-4)

The Center for Food Safety (0344) also pointed out that:

> "Oxytetracycline is being tested as a control for citrus greening disease and it could be sprayed in the future on citrus in Monroe County, FL and Harris County, TX." (Center for Food Safety 0344 p. 9) [Footnote omitted]

2. Sewage

Some comments (0342, 0344) offered suggestions on alternative routes through which tetracycline might be present in the test environment.

Friends of the Earth (0342) stated that:

> " . . , tetracycline is also a prevalent compound found in sewage, due to contamination from agricultural run-off and consumer disposal, for example. *Aedes aegypti* have been found to breed in sewage treatment plants, septic tanks, and cesspits in the Florida Keys. The possible widespread application and presence of tetracycline in the environment could significantly undermine the efficacy of GE mosquitoes to reduce overall mosquito populations. The 'female-killing' trait might fail if the mosquitoes are in

contact with sufficiently high levels of tetracycline." (Friends of the Earth 0342 p. 4) [Footnote omitted]

The Center for Food Safety (0344) added additional support for this comment pointing out that:

". . , a number of studies have found that *Aedes aegypti* mosquitoes can breed in septic tanks where there can be high levels of contamination with antibiotics such as tetracycline. A 2004 study found that sewage treatment plants, septic tanks, and cesspits were larval development sites for *Aedes aegypti* in the Florida Keys. In 2004, there were more than 36,000 septic systems and 5,000 to 10,000 cesspits in Florida." (Center for Food Safety 0344 p. 9) [Footnote omitted]

3. <u>Food, Food Waste and Animal Husbandry</u>

Some comments (0082, 0335, 0344) raised the possibility of food, food waste and animal husbandry being a source of tetracycline in the environment.

GeneWatch UK (0335) stated that:

"Ae. aegypti also commonly live in areas where discarded food is likely to contain meat contaminated with tetracycline; cat food would have sufficient amounts of tetracycline to keep the mosquitoes alive. Oxitec uses a diet supplemented with 30 µg/ml of the tetracycline to breed its OX513A mosquitoes in the lab: again, figures are not available for the OX5034 strain. The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow the GE mosquitoes to breed. Oxytetracycline can be found at concentrations above 500 µg/g in animal manure and doxycycline at up to 78516.1 µg/kg dry weight in broiler manure, which may be sufficient to inactivate the killing mechanism." (GeneWatch UK 0335 p. 7)

The Center for Food Safety (0344) added that:

"When OX513A GE mosquitoes were fed cat food containing industrially farmed chicken, which contains the antibiotic tetracycline, the survival rate increased to 15-18%. Oxitec . . . admitted to an 18% survival rate of larvae fed on cat food in a published paper. In the case of the OX5034 strain, no information has been provided whatsoever on the impacts of tetracycline on the likely survival rates of GE female mosquitoes." (Center for Food Safety 0344 p. 9) [Footnote omitted]

Anonymous (0082) stated that:

"We have heard the promises about how these GMO mosquitoes will produce sterile offspring and reduce the mosquito population and then discovered the the [sic] tetracycline from the antibiotics used in cattle ends up in the soil and allows for unanticipated reproduction." (Anonymous 0082 p. 1)

4. Comments Rebutting the Possibility that There may be Sufficient Tetracycline in the Environment to Allow Female *Ae. aegypti* Carrying the tTAV-OX5034 Gene to Mature to Adults

One commenter (0341) attempted to rebut concerns that there may be sufficient tetracycline in the environment to allow female Ae. aegypti carrying the tTav-OX5034 gene to mature into adults.

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) stated that:

> **"Environmental levels of tetracyclines high enough to help female OX5034 mosquitoes survive have never been recorded in the USA in potential *Aedes aegypti* breeding sites**, based on a comprehensive survey of the peer-reviewed literature (Meyer et al., 2000; Lindsey, Meyer & Thurman, 2001; Campagnolo et al., 2002; Yang & Carlson, 2003; Yang, Cha & Carlson, 2004, 2005; Kim et al., 2005; Karthikeyan & Meyer, 2006; Batt, Bruce & Aga, 2006; MacKie et al., 2006; Batt, Kim & Aga, 2007; Dolliver & Gupta, 2008b,a; Haggard & Bartsch, 2009; Kulkarni et al., 2017). The highest reported concentrations of environmental tetracyclines would be insufficient to allow survival of any female hemizygous OX5034 life-stages. The testing of antibiotic concentrations found in the environment is frequently associated with the efficacy of waste water treatment plants at removing antibiotics from waste water. Samples are taken from influent and effluent, and from rivers downstream of treatment plants. Antibiotic concentrations are also frequently tested in hog lagoons, which are anaerobic lagoons used to treat animal waste from farming pigs or other livestock. These are not typical breeding locations for *Ae. aegypti* larvae. *Ae. aegypti* is commonly referred to as a 'container breeding mosquito' as its preferred breeding sites include flower vases, tires, tree holes, etc. They are found in clean, still water, not flowing river systems and are rarely found in collections of water in the ground such as borrow-pits or earth drains (Christophers, 1960; Morrison et al., 2006; Dieng et al., 2012). Some reports have suggested that *Ae. aegypti* can breed in septic tanks (Barrera et al., 2008; Mackay et al., 2009) but this tends to be in the clear water at the top of the tank whereas tetracycline and their analogues tend to bind to the sediment which collects at the bottom (Brown et al., 2006; Watkinson et al., 2009). Therefore, the concentrations that resulted in functional female phenotypic rescue in this study are very unlikely to be found in typical breeding sites of *Ae. aegypti* (Curtis et al., 2015), therefore the potential for the efficacy of a control program using OX5034 to be compromised by the current reported levels of

environmental tetracycline and its analogues is negligible." (N. Rose 0341 p.8-9) [Emphasis in original]

C. Comments on the Potential Emergence of Resistance to OX5034 Trait or OX5034 Males

Some commenters (0038, 0039, 0226, 0317, 0329, 0331, 0335, 0344) were concerned about the possibility of evolution occurring either in the tTAV-OX5034 transgene or in the *Ae. aegypti* populations resulting in resistance to the self-limiting transgene or to the OX5034 releases. One commenter (0341) noted that no evidence of the emergence of resistance to the self-limiting gene had been observed during the numerous tests on OX513A.

The Florida Keys Environmental Coalition (0331) expressed concern about the possibility of evolutionary changes affecting the genetic material engineered into OX5034. To address this concern the Florida Keys Environmental Coalition (0331) requested that OX5034 be vetted by:

> "Lab trials for multigenerational survivability of genetic modifications (Oxitec published results are from computer models) and the outcome of evolutionary changes to manmade mutations, and verification of characteristics of any hybridized species. These long-term studies are based on expected mutations Mendelian genetic heredity, where errors occur causing evolutionary change. Given that the DNA of this species has been perturbed then evolution may, or may not, be more likely over a shorter time frame, due to instability caused by intended and unintended unnatural DNA sequences. Observation is warranted." (Florida Keys Environmental Coalition 0331 p. 3-4)

The Florida Keys Environmental Coalition (0331) also pointed out that EPA should investigate:

> " . . . of[f]-site [sic] unintended mutation caused by imprecise RNA transcripts. These assays are now much quicker and much less expensive, so standardizing these as part of a GM Species standardized regiment and evaluation is now practical and advisable." (Florida Keys Environmental Coalition 0331 p. 3)

The Florida Keys Environmental Coalition (0331) also stated that the following should be done prior to any open field release program in the US:

> "Mosquitoes should be evaluated with uncharacterized unintended mutations through full DNA sequencing." (Florida Keys Environmental Coalition 0331 p. 3)

Commenter B. Wray (0038) stated that:

> "Assays of a statistically significant sample set of the OX5034 should be performed to understand off target genetic mutations as part of the technical review to assure experimental use of the technology is safe." (B. Wray 0038 p. 2)

The Center for Food Safety (0344) stated that:

> "In addition, it is possible that the mosquitoes could develop a resistance to the lethality trait, which could lead to biting GE females being released into the environment. This further accentuates the EPA's need for a complete EIS and more thorough examination of unintended consequences before allowing Oxitec's application to be considered." (Center for Food Safety 0344 p. 9)

The Center for Food Safety (0344) noted that:

> ". . . resistance to the killing mechanism could evolve in the GE mosquito factory or in the environment." (Center for Food Safety 0344 p. 9)

GeneWatch UK (0335) stated that:

> "The percentage of surviving GE mosquitoes could also increase if resistance to the genetic killing mechanism evolves over time. In comparison, the traditional Sterile Insect Technique (SIT), used to control some pests, results in multiple chromosome breaks when the insects are exposed to radiation, severely limiting any potential for resistance to evolve during the production process. In contrast, any genetic or molecular event that allows the GE mosquitoes to survive and breed successfully could be rapidly selected for during mass production. Increased survival rates would reduce the effectiveness of any population suppression effect over time, increase the number of biting GE females, and potentially allow the GE mosquitoes to establish in the wild." (GeneWatch UK 0335 p. 7-8) [Footnotes omitted]

Anonymous (0226) stated that:

> "The percentage of surviving GM mosquitoes could also increase if resistance to the genetic killing mechanism evolves over time." (Anonymous 0226 p. 11) [Footnote omitted]

Anonymous (0329) added that:

> "GM mosquitoes could cause a huge increase in Aedes aegypti population if the lethality trait fails." (Anonymous 0329 p. 2)

Some commenters argued that the possibility of adaptive behavior ensuring the survival of offspring evolving in the Aedes mosquito population should be considered.

GeneWatch UK (0335) noted that:

> "In a conventional SIT programme in Japan, wild females appeared that were unreceptive to mating with irradiated males. Therefore, adaptive behaviour in wild females to increase survival of their offspring, including avoiding GE males or seeking out tetracycline-contaminated sites to lay their eggs, must also be considered." (GeneWatch UK 0335 p. 7-8)

Commenter D. Rubin (0317) referring to Evans et al, stated that:

> " . . . the effectiveness of the release program began to break down after about 18 months, i.e., the population which had been greatly suppressed rebounded to nearly pre-release levels. This has been speculated to have been due to mating discrimination against OX513A males, a phenomenon known to occur in sterile male release programs."

Anonymous (0039) noting that mutations occur all the time in the insect world, stated that:

> "As in the wohlbaccia [sic] deal, it's been found that certain insects change sex in order to keep the species going!!!" (Anonymous 0039 p. 1)

However, N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) stated that:

> " . . . , most sterile male release programs (whether insects are sterilised by irradiation, as for screwworm, fruit flies or other mosquitoes, or by genetics) have recorded no evidence of assortative mating (Alphey et al., 2010), which states "*Resistance through assortative mating has been reported in several cases, but was generally found to be associated with a loss of quality in the mass-reared insects, probably due to inbreeding; this was rapidly reversed by improved genetics. We know of only one instance of reasonably well-documented resistance through assortative mating* (McInnis, Lance & Jackson, 1996) *in the absence of clear decline of sterile insect production quality in the entire 50+ years of SIT*." (N. Rose, 0341 p. 5)[Emphasis in the original]

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) added that:

> "Selective mating has never been observed in any releases of close to 1 billion Oxitec males worldwide." (N. Rose 0341 p. 4)

EPA Response to Comments in Part VI.A. – Comments Questioning the Penetrance of the OX5034 Trait and Positing that Contamination of Adult Male OX5034 Mosquito Releases by Adult OX5034 Females Could Occur. Several comments referred to data from the OX513A mosquito which is a different transgenic mosquito developed by Oxitec and is not covered under the current EUP application. The sex sorting and pesticidal effect of the OX513A mosquito is fundamentally different from that reviewed in the current application for the OX5034 mosquito, as OX513A product used pupal size sorting to separate males and females and had a <~5% larval survival rate regardless of larval sex. Conversely, the pesticidal effect of the OX5034 mosquito is female specific and results in 100% lethality of OX5034 female mosquitoes in the absence of tetracycline. The female specific lethality of the trait is used for sex sorting to ensure that only OX5034 male mosquitoes are released into the environment, thereby eliminating the need for pupal size sorting to separate males and females.

EPA has carefully evaluated Oxitec's claim of 100% female lethality in the Human Health and Environmental Risk Assessment (EPA-HQ-OPP-2019-0274). EPA evaluated data from laboratory crosses of OX5034 mosquitoes and wild-type mosquitoes that demonstrated that no OX5034 females reached the adult stage when reared without the tetracycline antidote, confirming 100% penetrance of the OX5034 trait. Importantly, complete penetrance was also demonstrated in OX5034 hemizygous females, which would be genetically comparable to the female offspring that would result in the field from OX5034 male releases. Additional data were also provided examining penetrance of the OX5034 trait from field trials that occurred outside of the United States. These data confirmed that the OX5034 trait is 100% penetrant in the field when expressed in a geographically distinct genetic background. It is therefore concluded that the OX5034 mosquito does indeed demonstrate 100% penetrance in terms of female lethality.

EPA Response to Comments in Part VI.B. – Comments Questioning Whether Sufficient Tetracycline Occurs in the Test Environment to Allow Tetracycline-Dependent Female *Ae. aegypti* Mosquitoes to Mature to Adults. EPA agrees with the commenters that tetracyclines can be found in the environment and can come from human or animal drugs, or non-drug sources such as in agriculture. As such, EPA carefully evaluated in the Human Health and Environmental Risk Assessment, the likelihood that OX5034 mosquitoes would encounter tetracycline sources at levels high enough to rescue females from the lethal trait.

Testing of the dose-response of OX5034 mosquitoes to a range of tetracycline analogues was evaluated to determine levels required to rescue female OX5034 mosquitoes. These rescue levels were compared to concentrations of the tetracycline analogues typically found in the environment as a result of industrial or household tetracycline usage in the United States. In all cases the minimum concentration for each analogue required to rescue OX5034 females capable of maintaining flight is at least two times higher than the mean concentrations found in environmental water bodies for the studies reviewed. EPA also considered potential sources of tetracycline specific to the proposed trial areas and concluded the likelihood that OX5034 mosquitoes would encounter tetracycline levels high enough to result in OX5034 females is low. The Human Health and Environmental Risk Assessment arrived at this conclusion based on

known oviposition preference of *Ae. aegypti* and literature surveys of environmental concentrations of tetracycline analogues indicating levels lower than those shown necessary through dose response testing to rescue OX5034 females. By further reducing access to potential tetracycline sources (i.e., wastewater treatment facilities and citrus groves) through limiting proximity of the outer trial boundaries to such potential sources, the likelihood will be reduced to negligible.

With regard to agricultural sources of tetracycline, given that the proposed trial areas are likely to be in relatively developed (urbanized) areas due to preferred *Ae. aegypti* habitat, the presence of livestock or aquaculture is not expected. However, because Florida is a major producer of citrus and oxytetracycline applications are being used in citrus groves to combat citrus greening, the outer boundary of the trial areas will be greater than 500 m from commercial citrus growing areas to reduce the likelihood that OX5034 mosquitoes could encounter increased levels of oxytetracycline as a result of these applications.

With regard to sewage sources of tetracycline, commenters pointed out that a 2004 publication identified that *Ae. aegypti* breed in sewage treatment plants, septic tanks, and cesspits in the Florida Keys. However, since 2004, Key West and surrounding areas in Monroe County have eliminated most septic tanks and use a public sewer line system as the major means of waste disposal. Each of the Florida Keys Aqueduct Authority Wastewater Districts has its own municipal wastewater treatment facility, which consist of a series of open holding tanks that could be used as a breeding ground, although the likelihood of these tanks containing high enough levels of tetracycline to rescue OX5034 females is also low because tetracycline rapidly undergoes aqueous photolysis in the presence of sunlight. In Harris County, Texas, reclaimed water from all of Houston wastewater plants is discharged directly into a surface waterway, usually one of the area bayous, which are not typical breeding sites for *Ae. aegypti* and any tetracycline present would also undergo aqueous photolysis. However, the outer boundary of the trial areas will be greater than 500 m from any wastewater treatment facility in either Monroe or Harris County to further reduce the likelihood that OX5034 mosquitoes could encounter increased levels of tetracycline from sewage sources.

With regard to food, food waste, and animal husbandry sources of tetracycline, given that the proposed trial areas are likely to be in developed (urbanized) areas due to preferred *Ae. aegypti* habitat, the presence of livestock or aquaculture is not expected. Thus, cattle, chicken or other husbandry animal manures, which may contain pass-through antibiotics, are not expected to be present in significant quantities. However, a commenter noted a laboratory study in which, when OX513A larvae were exclusively fed a chicken-based cat food, some survival to adulthood occurred due to tetracycline contamination. As the trial areas are expected to be in urbanized areas, the presence of pets and their food, such as cat food that may originate from organs/meat from antibiotic treated husbandry animals, is likely. However, cat food is not believed to be a plausible source of tetracycline exposure for OX5034 mosquitoes in the environment as it would require that adequate levels of tetracycline would be found in the cat food. This would require a number of steps: that the cat food be found in a container to create

a high enough concentration of tetracycline to rescue OX5034 females, that the container also hold adequate levels of water for mosquito development, and that these conditions be maintained over a period of at least 8-10 days for larval and pupal development. In addition, exposure to sunlight would result in aqueous photolysis, so to maintain adequate tetracycline levels the cat food container would have to remain in the shade. For the reasons cited for cat food, other meat-based pet foods are not considered to be plausible sources of tetracycline exposure.

EPA Response to Comments in Part VI.C. – Comments on the Potential Emergence of Resistance to the OX5034 Trait or OX5034 Males. With regard to comments expressing concern about the possibility of off-target or other mutations, the commenters offered no information or explanation for these concerns. Nor have the commenters offered any suggestion as to what types of changes might be monitored for, nor why such changes, if they occurred, might be significant. While it is possible to sequence a mosquito genome, genomic sequences can vary from individual to individual in a population. Thus, even within the local wild *Ae. aegypti* population, a range of variation exists in the genes and in other genetic material of the genomes of individuals in that population. Full genome sequencing in and of itself thus is unlikely to provide information useful in a risk assessment.

With regard to comments expressing concern about the possibility of evolutionary changes affecting the genetic material engineered into OX5034, the commenter offered no information or explanation on how such evolutionary changes might occur or the significance of such changes should they occur. Spontaneous mutations naturally occur in organisms and contribute to genetic diversity. While unlikely, resistance to the OX5034 lethal trait may develop through mutations of genetic elements that are associated with the self-limiting function. Mutations in conserved parts of the cellular machinery that are necessary for the lethal effect to occur would be expected to carry a significant fitness cost for the individual as other essential functions of the cell would likely also be affected. Additionally, only a subset of mutations in the genetic cassette would have the potential to affect the function of the positive feedback loop in a meaningful way. The company reports that genetic resistance to the OX5034 trait has not been observed in 27 generation equivalence of OX5034 nor as part of the field releases involving over 12 million OX5034 homozygous males and during the EUP, Oxitec will continually monitor for the occurrence of genetic resistance. Considering these lines of evidence together, the likelihood for genetic resistance to occur during the field releases is negligible.

With regard to behavioral resistance where wild females might evolve to preferentially mate with wild males rather than OX5034 males, resistance through assortative mating has not been a common occurrence in other modified insect release programs. Although there is the potential for resistance through this mechanism in *Ae. aegypti* as there is evidence for rapid evolution in mating preference in this species, the impact on humans or non-target organisms would be negligible as this would not result in a new risk or exposure scenario. Instead, if behavioral resistance were to evolve, it would result in decreased efficacy of the OX5034 releases through lack of successful matings. The reduction in efficacy would not pose an

increased risk from nuisance biting or disease vectoring from the local *Ae. aegypti* population because similar mosquito abatement activity will occur in both treated and untreated areas during the proposed EUP.

VII. Comments on Human Health Considerations

Comments on the potential for OX5034 to affect human health revolved around potential for: (1) the spread of viral pathogens by OX5034 mosquitoes; and (2) the proteins engineered into OX5034 to be toxic or allergenic.

A. Comments on Potential for Disease Transmission from OX5034 mosquitoes

Some commenters (0223, 0226, 0335, 0342, 0344) expressed concern that any female *Ae. aegypti* mosquitoes that might result from the EUP releases might vector disease. Commenters said the following should be considered: (1) already infected females might be released during the testing; and (2) pathogenic viruses could evolve in response to OX5034.

1. Comments Raising Concerns that Virus Infected Females Might be Released During Testing

Some commenters (0335, 0342, 0344) expressed the concern of a possibility that virus infected female OX5034 mosquitoes might be released during testing.

Friends of the Earth (0342) stated that experience shows there could be a potential for biting female *Ae. aegypti* mosquitoes to be released during the testing:

> "Oxitec's initial Draft EA to the FDA about the OX513A mosquitoes acknowledges that it is inevitable that some biting female GE mosquitoes will be released. Similarly, GE female OX5034 mosquitoes, which can bite and transmit disease, could be released into the environment during the experiments." (Friends of the Earth 0342 p. 5)

GeneWatch UK (0335) and the Center for Food Safety (0344) went on to caution that "the possibility that the released GE mosquitoes are already infected with diseases also needs to be considered":

> "Oxitec's draft Environmental Assessment for its OX513A strain, as submitted to the FDA (page 31), stated that the horse blood it uses to feed the GE mosquitoes at its UK production facility is screened for equine infectious anemia (EIA) and equine viral arteritis (EVA) among other pathogens, to minimize the potential for contamination of the blood by virus, bacteria, or other pathogenic agents. It also notes that the host

> range of *Aedes aegypti* and *Aedes albopictus* does not extend to the UK, so the risk of transmission of arbovirus such as dengue and chikungunya to these horses is negligible. However, the range of *Aedes albopictus* has been expanding in Europe and there have been warnings that this vector could reach the UK in future. The UK has several endemic mosquito species (mainly *Culex* species) that could potentially act as vectors for West Nile Virus in the future. It is also unclear what feed source Oxitec intends to use in its US rearing facilities. To reduce the risk that infected mosquitoes (potentially including some biting females) are released, a protocol for testing the GE mosquitoes for pathogenic agents should be introduced at the proposed rearing facilities. Up-to-date information regarding the feeding of the OX5034 strain also needs to be provided." (GeneWatch UK 0335 p. 6; Center for Food Safety 0344 p. 8) [Footnotes omitted]

Friends of the Earth (0342) urged that EPA should ensure that there are testing protocols in place to prevent release of infected OX5034 mosquitoes:

> " . . . and the EPA should ensure that there is a stated protocol for testing the GE mosquitoes for pathogenic agents at the proposed rearing facilities." (Friends of the Earth 0342 p. 5)

The Center for Food Safety (0344) and GeneWatch UK (0335) stating that there "are no published peer-reviewed paper for Oxitec's GE *Aedes aegypti* OX5034 mosquitoes" indicated that necessary tests included:

> "A protocol for testing the GE mosquitoes for pathogenic agents prior to release." (GeneWatch UK 0335 p. 15-16)

2. Pathogenic Viruses Could Evolve in Response to OX5034

A few commenters (0223, 0226, 0335, 0342, 0344) questioned whether the arboviruses transmitted by *Ae. aegypti* could evolve in response to OX5034 releases.

Friends of the Earth (0342) stated that:

> "Lastly, there is concern around the possibility of the dengue virus to evolve and become more potent and virulent in response to the introduction of the GE mosquitoes, and this could put human health at greater risk". (Friends of the Earth 0342 p. 5) [Footnotes omitted]

Friends of the Earth (0342) echoed that statement, calling for an assessment that looked at:

> "Potential adverse impacts that the release of GE mosquitoes may have on the ability of dengue fever to evolve and become more virulent." (Friends of the Earth 0342 p. 7)

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that an assessment should include studies addressing:

> " . . . the possibility that viruses will evolve in response to ecosystem changes." (GeneWatch UK 0335 p. 16; Center for Food Safety 0344 p. 18)

T. Ritchie (0223) stated concern that in Brazil post OX513A testing, frequency of occurrence of diseases vectored by *Ae. aegypti* mosquitoes did not decrease:

> "I know that there has been some conflicting news on this experiment so I had decided to go straight to a source of information, a Brazilian newspaper. This was to conclude as to whether or not the experiment worked in Brazil where the altered mosquitoes had been released in 2016. This paper is from Sao Palo, Brazil on September 11, 2019 and they are saying that there is an INCREASE of all three illnesses by Aedes aegypti mosquitoes: dengue, chikungunya, and our most feared zika. They have reported the statistics: " The number of cases of dengue, zika and chikungunya, diseases transmitted by the mosquito Aedes aegypti, increased in Brazil this year. In all, the three diseases caused 650 deaths as of December 30, 2018 and August 24, 2019. The Southern region had the highest percentage increase in new cases of the three diseases. The largest percentage increase was registered by dengue cases, a jump of 599.5%. As of August 24, there were 1,439,471 cases diagnosed in Brazil - or 690.4 cases per 100,000 inhabitants - and 591 deaths. In So Paulo, the state with the sharpest increase, the number of cases is 38 times higher than the previous year (3,712%), jumping from 11,475 to 437,047 cases. In Paran, the jump was 3,563%. Already cases of chikungunya went from 76,742 last year to 110,627 in 2019, registering a rate of 53.1 cases per 100,000 people. In the case of chikungunya, the state with the highest percentage change was Alagoas, 1,011%, a jump from 138 cases to 1,534. Regarding cases of Zika, the growth registered in Brazil was 47.1%, with 9,813 and 2 deaths. The incidence rate is 4.7 cases per 100 thousand inhabitants. The state with the largest percentage increase was Rio Grande do Sul, 1,083%. . . . Please do not release the GMO mosquitoes in the states of Florida and Texas. We really don't want to have an increase of mosquitoes and their illnesses like Brazil has!" (T. Ritchie 0223 p. 1-2) [Emphasis in the original]

Anonymous (0226) stated that:

> "Perhaps the most important issue is whether cases of the more serious dengue haemorrhagic fever might increase as a result of the experiments. In its draft risk assessment submitted to regulators in the USA Oxitec states: *' It has been suggested that in countries with very high transmission rates, reduction in transmission could*

increase the frequency of dengue haemorrhagic fever (DHF) even while decreasing the incidence of dengue fever.' The mechanism is a possible loss of cross-immunity to multiple serotypes of dengue." (Anonymous 0226 p. 11) [Emphasis in the original] [Footnotes omitted]

Anonymous (0226) added that:

> "Another possibility is that there is a rebound in number of dengue cases due to loss of human immunity." (Anonymous 0226 p. 11) [Footnotes omitted]

B. Comments on Toxicity/Allergenicity of Proteins Engineered into OX5034

Some commenters (0181, 0306, 0329, 0335, 0342, 0344) expressed concern that the proteins engineered into OX5034 *Ae. aegypti* mosquitoes could pose risk to human health through toxicity or allergenicity given the potential for exposure to the proteins through two possible routes - ingestion of the mosquitoes and injected mosquito saliva.

1. Potential for Human Exposure Through the Oral Route

Some commenters (0335, 0342) suggested that humans could be exposed to *Ae. aegypti* mosquitoes expressing the tTAV-OX5034 and/or DsRed2-OX5034 proteins through the oral route, i.e., through ingestion of either larvae of flying adults.

Friends of the Earth (0342) stated that:

> "Given the high number of mosquitoes that are proposed for release, and based on experience in the Brazil, there is a high likelihood that humans or animals could swallow the GE mosquitoes upon release. As reported in Brazil, because of the high number of GE mosquitoes released, "… it's impossible to talk during the liberation sessions without accidentally swallowing a few…" (Friends of the Earth 0342 p. 4-5) [Footnote omitted]

Similarly, GeneWatch UK (0335) stated that:

> "Humans, animals and wildlife will also swallow adult GE mosquitoes. Journalists have reported that in Brazil, during experiments with Oxitec's OX513A GE mosquitoes, "…*it's impossible to talk during the liberation sessions without accidentally swallowing a few*…" due to the very large numbers of GE mosquitoes being released to try to swamp the wild population". (GeneWatch UK 0335 p. 13-14) [Emphasis in the original]

GeneWatch UK (0335) also pointed out that:

> "Because the female GE mosquitoes mostly die at the larval stage, there will be large numbers of dead GE larvae in the water where the female mosquitoes lay their eggs, and these might be ingested by humans, animals or wildlife." (GeneWatch UK 0335 p. 13-14)

2. Potential Human Exposure Though the Saliva of the Biting Female Mosquito

Some commenters (0181, 0306, 0335, 0342) suggested that humans could be exposed to OX5034 proteins through the bite of a female *Ae. aegypti* mosquito.

GeneWatch UK (0335) commented that:

> " . . , people and animals may be bitten by female GE mosquitoes, if any survive or are inadvertently released." (GeneWatch UK 0335 p. 14)

Friends of the Earth (0342) stated that:

> "Also of concern is that biting female GE mosquitoes may inject a novel engineered protein into humans; Oxitec has yet to conduct or publish any study showing that this novel protein is not expressed in the mosquito's salivary gland." (Friends of the Earth 0342 p. 4-5)

Commenter M. Hull (0306) stated that:

> "According to Oxitec, a percentage of OX5034 will be females, which will bite warm blooded mammals, and their saliva will mix with the blood of those warm blooded mammals, exposing victims to foreign, experimental DNA sequences." (M. Hull 0306 p. 1)

Anonymous (0181) asked why the Agency would even consider:

> " . . . allowing mosquitoes that will produce toxins and poisons in your own human body into the environment?"(Anonymous 0181p. 1)

3. Comments on Toxicity

Some commenters (0003, 0318, 0329, 0335, 0344) expressed concern that the proteins engineered into OX5034 might be toxic. One commenter (0343) argued that the proteins engineered into OX5034 are not toxic.

Anonymous (0329) stated that:

> "GM mosquito expresses protein that has been shown to be toxic to more than just the *Aedes aegypti*. The tTA protein has been shown to be a toxin and neurotoxin to rodents, which are mammals, and therefore may be toxic to other mammals such as bats or even humans that might consume them. Signs of toxicity and neurotoxicity have been reported in mice expressing the tTA protein. Other mice studies have detected adverse effects on the lung." (Anonymous 0329 p. 1)

Further, GeneWatch UK (0335) stated that:

> "In the scientific literature, there is some evidence that enhanced tTAV expression can have adverse effects (loss of neurons affecting cognitive behaviour) in transgenic (GE) mice. Other mice studies have detected adverse effects on the lung. These studies should act as warning signs that further evidence is needed." (GeneWatch 0335 p. 14) [Footnote omitted]

Anonymous (0003) stated that:

> "I don't believe this protein should be expressed in Florida or Texas. We have problems with proteins causing problems with Creutzfeldt and with chronic wasting disease that are ruining and injurious to brains. It is best not to allow this. It could be quite injurious." (Anonymous 0003 p. 1)

GeneWatch UK (0335) and Center for Food Safety (0344) stated that more information on the proteins engineered into OX5034 is needed:

> "However, other than a bioinformatics report (desk study), Oxitec has to date provided limited evidence regarding the safety of the RIDL genetic mechanism and the high level expression of tTAV that kills the insects at the larval stage. The mechanism of action of this killing mechanism is not fully understood and very limited safety data is available. The tetracycline transactivator (tTAV) protein is created by fusing one protein, TetR (tetracycline repressor), found in *Escherichia coli* bacteria, with the activation domain of another protein, VP16, found in the Herpes Simplex Virus. Researchers commonly use this mechanism to switch on and off different genetic traits, for example in transgenic (GE) mice used in medical research, but it is not normally present in the human food chain." (GeneWatch UK 0335 p. 14; Center for Food Safety 0344 p. 16)

Commenter J. Rubin (0318) stated that:

> "According to Science Magazine, even Oxitec does not completely understand their living creation: ""But the gene Oxitec uses, known as tTAV (tetracycline-repressible transcriptional activator variant) is designed to drive the expression of even more tTAV in what becomes a fatal feedback loop. "How the process kills mosquitoes isn't entirely clear; the excess tTAV protein may tie up the cell's protein production machinery. "It basically causes genetic havoc, and the organism dies," says Al Handler, an insect geneticist at the U.S. Department of Agriculture in Gainesville, Florida." https://www.sciencemag.org/news/2016/10/brazil-will-release-billions-lab-grown-mosquitoes-combat-infectious-disease-will-it?fbclid=IwAR2z-PIluYvqZtcdp1xaXu6u-apgfv7l2POJ5OrJ7M28U8NOgqBD5lEL_l4 So, if Oxitec does not completely understand its creation, how can EPA understand it and all of the implications a release of these genetically modified organisms into our state and our delicate, beautiful ecosystem will have?" (J. Rubin 0318 p. 2)

On the other hand, J. Morris, International Center for Law & Economics, (0343) stated that:

> "The self-limiting gene, tTAV (tetracycline repressible transactivator variant), is a gene variant that has been optimized to work only in insect cells. In the wild, offspring that contain the self-limiting gene make a non-toxic protein that ties up the cell's machinery, so its other genes are not expressed and the insect dies. The proteins produced are non-toxic in the insects, so if any animals eat them it would be the same as eating a wild insect – they are digested in just the same way that all other insects are digested." (J. Morris, International Center for Law & Economics, 0343 p. 6) [Footnote omitted]

4. Allergenicity

Several comments (0066, 0232, 0329, 0331, 0335, 0344) were received questioning whether the introduced tTAV-OX5034 and DsRed2-OX5034 proteins could be allergens. Two types of comments were received on allergenicity potential: comments that expressed concern and those that express reassurance about safety. One commenter (0337) shared his opinion that these proteins were not allergens.

i. Comments expressing concern

Anonymous (0329) stated that:

> "The proteins expressed by GM mosquitoes contain amino acid sequences identical to known human allergens." (Anonymous 0329 p.1)

Florida Keys Environmental Coalition (0331) stated that:

> "If females with modification are detected in measure statistical study, then allergen studies should be performed for these insects and clinical trial of the bite on humans must be performed. Oxitec has been negligent in performing any studies that evaluate the effect of the OX 513A bite and the same lack of knowledge should be avoided for the OX5034." (Florida Keys Environmental Coalition 0331 p.3)

GeneWatch UK (0335) and Center for Food Safety (0344) indicated that some information that might potentially be of relevance to the DsRed2 protein is available, but even this information is limited:

> "In its application to release GE moths in New York State (since withdrawn but later resubmitted, although a brief open-release trial has now ceased), Oxitec provides a commercial reference for toxicity testing of the red fluorescent marker, DsRed2, by Pioneer DuPont." (GeneWatch UK 0335 p. 14; Center for Food Safety 0344 p. 16) [Footnote omitted]

GeneWatch UK (0335) and Center for Food Safety (0344) added that there is also some limited information available on a related protein, green fluorescent protein (GFP):

> "Oxitec also cites a 26- day feeding study in rats, using GE oil seed rape (canola) genetically modified to express green (not red) fluorescent protein (GFP), which concludes: "*These data indicate that GFP is a low allergenicity risk and provide preliminary indications that GFP is not likely to represent a health risk*". (GeneWatch UK 0335 p. 14; Center for Food Safety 0344 p. 16) [Emphasis in the original] [Footnote omitted]

Commenter B. Vaughn (0232) questioned whether enough is known about the mosquitoes to be sure they are safe. He stated:

> "Do we know whether or not they will carry or develop any unknown pathogens that can hurt humans? I know that my daughter was bitten by what we just thought was a mosquito when we were in Florida back in the Summer of 2017, her face had a severely negative reaction. We started researching and calling authorities in Florida, trying to figure out what could have happened. It couldn't have been a mosquito based on that reaction, so what could it have been? She had never reacted like this before, and that has set off a series of one after another, after another negative reactions we've seen in her since. It's been hell. Now, I'm reading that millions of GMO mosquitoes were released in Florida, just a couple of months before that, in May 2017." (B. Vaughn 0232) p. 1)

Commenter C. Harman (0066) opposed release of mosquitoes, including because:

> "In addition, mosquito bites can cause severe skin irritation through an allergic reaction to the mosquito's saliva - this is what causes the red bump and itching." (C. Harman 0066 p. 1)

ii. Comments expressing reassurance

R. E. Goodman (0337) stated that he had:

> " . . . evaluated potential allergenicity and toxicity of the first generation, OX513A, using bioinformatics as one would do for a GM plant, microbe or animal according to CODEX Alimentarius guidelines. There was no significant sequence to allergens or toxins, and that is the primary potential risk factor, identity matches to a known allergen or toxin. I have not worked on the second generation, but as it is described, I assume the proteins are still the same." (R. E. Goodman 0337 p.1)

EPA Responses to Comments in Unit VII.A. – Comments on Potential for Disease Transmission from OX5034 mosquitoes. The OX5034 mosquito relies on a genetic sex-sorting mechanism that has demonstrated 100% female lethality. The genetic sex-sorting mechanism results in no OX5034 females being released and no OX5034 female offspring surviving to adulthood in the absence of tetracycline. EPA evaluated the likelihood of arboviral infection in the OX5034 colony in the Human Health and Environmental Risk Assessment found in the docket established for this action (EPA-HQ-OPP-2019-0274).

Briefly, the four viruses that have had the greatest impact on human health for which *Ae. aegypti* is the primary vector include the Yellow Fever Virus, Dengue Viruses, Chikungunya Virus, and Zika Virus. The homozygous OX5034 *Ae. aegypti* colony is maintained in a dedicated insectary in the United Kingdom (UK) that complies with biological containment level 2 standards. The dietary horse blood provided to females to enable egg production is sourced from a closed, British herd. The herd is under veterinary care, tested for certain equine viruses, and each blood batch is tested by the supplier for bacterial sterility. At this time, neither *Ae. aegypti* nor the major viruses it transmits are established in the UK. It is therefore unlikely that these viruses are present in the OX5034 mosquito colony.

With regard to comments stating the need for a testing protocol, the Agency concludes that because neither *Ae. aegypti* nor the arboviruses for which it is a vector are present in the UK, the risk of infection of arbovirus infection of the source colony is low. Therefore, arbovirus testing for the EUP is not required.

With regard to comments discussing the potential for viral evolution in response to OX5034 mosquitoes, the publication cited by commenter 0342 states that control strategies that result in mosquito mortality (such as the OX5034 mosquito) actually have a reduced risk of causing increase virulence than other control strategies.

With regard to comments discussing the potential for an increase in disease prevalence or loss in human immunity to disease due a reduction in the mosquito population, the commenters did not provide sufficient information in the context of this EUP request to enable EPA to respond to the comments.

EPA Response to Comments in Unit VII.B. – Comments on Toxicity/Allergenicity of Proteins Engineered into OX5034. The Human Health and Environmental Risk Assessment found in this docket (EPA-HQ-OPP-2019-0274) evaluated issues raised in comments in Unit VII.B of this Response to Comment document. EPA's human health risk assessment evaluated whether the tTAV-OX5034 or DsRed2-OX5034 proteins could be toxic or allergenic through either the oral or dermal route of exposure. At this time the Agency has not determined whether the results of the presented protein homology and literature-based assessments alone are sufficient to support the hazard assessment of tTAV-OX5034 and DsRed2-OX5034. Due to the lack of females, i.e., negligible exposure, the risk from OX5034 is considered negligible and thus, the hazard data were not necessary to support the finding of no unreasonable adverse effects for humans. EPA's risk assessment also concluded that the female-killing trait engineered into OX5034 resulted in 100% mortality for female *Ae. aegypti* mosquitoes carrying a copy of the tTAV-OX5034 gene (also addressed in Unit VI. above). Thus, as there will be no adult female OX5034 *Ae. aegypti* mosquitoes in the test area and as only adult female mosquitoes bite, EPA has concluded there is a negligible likelihood of dermal exposure to the tTAV-OX5034 and DsRed2-OX5034 proteins through the saliva in a bite from a female OX5034 mosquito. The only other potential route of dermal exposure to these proteins would be if a male OX5034 mosquito should alight on the bare skin of a human, and the human crushed the mosquito onto the bare skin. However, given that males do not feed on humans, the frequency of human interaction with male mosquitoes is expected to be minimal. Even if direct skin contact were to occur, because the tTAV-OX5034 and DsRed2-OX5034 proteins are present within the insect's cells, exposure to these substances is expected to be negligible. (see Unit II.A.2. "Pesticidal activity of the active ingredient tTAV-OX5034" in the Human Health and Environmental Risk Assessment found in this docket).

With regard to the comment on the existence of literature reports that the tTA protein may be a neurotoxin or have adverse effects on the lungs of transgenic mice, tTA proteins can exert an adverse effect when engineered into an animal. However, these effects are generally thought to be the result of differential gene expression mediated by the tTA proteins and there is no indication from the literature that these effects are due to an inherent toxicity of the protein (see Unit II.B.2. "Mammalian toxicity and allergenicity assessment" in the Human Health and Environmental Risk Assessment found in this docket).

With regard to the comment equating the tTAV-OX5034 protein to proteins that can form the prions believed to be causative factors in Creutzfeldt-Jacob and chronic wasting diseases, the commenter did not provide sufficient information to support such an argument. A prion is an abnormal form of a normally harmless naturally occurring brain protein that has the ability to

transmit its misfolded shape onto normal variants of the same protein. In contrast, as noted above, the pesticidal activity of the tTAV-OX5034 protein is dependent upon the tTAV-OX5034 gene being engineered into the animal and expressed intracellularly.

With regard to the comment that millions of GMO mosquitoes were released in Florida in May 2017, no releases of genetically engineered mosquitoes have occurred in the United States to date.

VIII. Comments on Environmental Considerations

Comments on environmental considerations revolve around the potential for releases of OX5034 mosquitoes to have effects on other organisms. Such effects could be direct such as through consumption of OX5034 mosquitoes or indirect through dynamic changes in the ecosystem. Comments discussed whether such dynamic interactions could occur between *Ae. aegypti* and *Ae. albopictus* populations as well as between *Ae. aegypti* and other mosquito species. Commenters also questioned whether such dynamic changes could affect other organism populations. One commenter analogized release of OX5034 mosquito to the accidental introduction of Africanized Bees to the American continents. Commenters also requested additional testing be performed prior to releases.

A. Effects on Food Supply

Comments pointed out that OX5034 releases could affect animals in the area of the release, primarily through effects on the food supply of organisms in the test area, such as a loss of food supply or fluctuations in their food supply. EPA received 15 comments on the possibility of effects on the food supply of organisms in the test area. (0014, 0041, 0043, 0052, 0062, 0110, 0173, 0205, 0208, 0264, 0308, 0314, 0318, 0335, 0344).

Commenter A. Purkis (0308) stated that:

> "Though the mosquito is an introduced species, native species are now reliant on these mosquitoes for their diet." (A. Purkis 0308 p. 1)

Anonymous (0110) stated that:

> "Our mosquitos, however undesirable they may be, are food for birds and other insects. If we manipulate the food chain, at what point could it directly result in an unforeseeable and possibly irreversible change that the world is not prepared to deal with." (Anonymous 0110 p. 1)

Anonymous (0043) stated that:

> "Don't mess with Mother nature. You don't know the consequences of your "experiments". What happens to the food chain? Birds, lizards and Dragonflies?" (Anonymous 0043 p. 1)

Commenter J. Berman Diaz (0264) stated that:

> "Many other species eat mosquitoes- what happens when frogs consume these GMOs. GMO safety has NOT been proven!" (J. Berman Diaz 0264 p. 1)

Commenter J. Rubin (0318) stated that:

> "How will bats be affected? Bats are already facing serious population declines. Many bats, and almost all in the United States, thrive on an insect diet. A single bat can eat up to 1,200 mosquito-sized insects every hour, and each bat usually eats 6,000 to 8,000 insects each night. Bats play an important role in many environments around the world. Some plants depend partly or wholly on bats to pollinate their flowers or spread their seeds. Has EPA explored how bats may be affected by Oxitec mosquito releases?" (J. Rubin 0318 p. 2)

Anonymous (0173) stated that before any pesticide is used:

> "It must be also proven there are no adverse effects to pollinators such as bees and butterflies." (Anonymous 0173 p. 1)

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that any analysis must take into account that the ecosystem will react dynamically when large numbers of male *Ae. aegypti* mosquitoes are released into it. The Center for Food Safety indicated for example that:

> ". . . , any assessment of the potential impact on the environment of the proposed releases must consider more than the desired reduction in the *Aedes aegypti* population in the release area on wild animals that may feed on them. In reality, there will be a very large increase (several orders of magnitude) in *Aedes aegpti* numbers (largely GE adult males, but perhaps also spikes in adult females, . . .) in the target area during the releases, and potential increases in surrounding areas (possibly including large numbers of wild males if they migrate from the release site to avoid competition with the GE males that are released). This may be followed by a fall in wild numbers at the release site if the experiment is successful in achieving population suppression, and perhaps a subsequent rebound if the mosquitoes evolve resistance or begin to breed in tetracycline-contaminated sites, or if continued releases become technically difficult or

> uneconomic. Consideration of the impacts requires consideration of a dynamic ecosystem that may respond in complex ways. . . . Oxitec's treatment of this issue to date has been inadequate because it does not consider the complex and dynamic nature of the ecosystem." (Center for Food Safety 0344 p. 13 GeneWatch UK 0335 p.11) [Emphasis in original]

The Center for Food Safety (0344) stated that:

> ". . . , species that feed on mosquitoes may initially be attracted to the site, but lose access to the new food supply as the numbers of the target species at the site reduce." (Center for Food Safety 0344 p. 13)

Center for Food Safety (0344) stated that:

> ". . . , species which feed on adult *Aedes aegpti* [sic] are likely to have an increased proportion of this species in their diets, due to the need to swamp wild males by several orders of magnitude during the releases . . . ." (Center for Food Safety 0344 p. 13)

B. Population Dynamics

Several comments (0226, 0329, 0335, 0342, 0344) were received addressing population dynamics. Comments revolved around three concerns: (1) the releases of OX5034 male mosquitoes might result in an increase in numbers of mosquitoes in areas surrounding the test site; (2) *Ae. albopictus* might competitively displace *Ae. aegypti*; and (3) Culex species might also be affected by the release of large numbers of *Ae. aegypti* males.

Friends of the Earth (0342) stated that:

> "Oxitec's application does not consider the complexity of ecosystems carefully enough. A complete EIS should not only look at the risks from one release, but the potential impacts of releasing millions of mosquitoes on a continual basis, and whether the proposed experimental use will cause unreasonable adverse effects on the environment." (Friends of the Earth 0342 p. 3)

1. Increases in Wild Mosquito Numbers in Surrounding Areas

A few comments (0335, 0344) were concerned that because of the large number of male mosquitoes released during the testing, local wild mosquitoes might migrate out of the test site to surrounding areas.

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "Another issue is whether or not releases of GE mosquitoes could cause an increase in the numbers of mosquitoes in surrounding areas. This effect is predicted by some models for the release of sterile insects. There is evidence from Oxitec's experiments with its OX513A strain that numbers in neighbouring control areas may increase as the population is suppressed in the target area. For example, in its 2009 Cayman Islands experiments, the number of wild *Aedes aegypti* mosquito eggs, measured using egg traps (ovitraps), was observed to increase in the neighbouring control area as the population in the release area decreased (Figure 2c). The same effect can be seen in Oxitec's experiments in Itaberaba (Brazil), which compare ovitrap data from the control area with data from adult male traps in the release area (Figure 2D). Thus, there appears to be a real possibility that some of the wild mosquitoes, when swamped by very high releases of GE males, simply migrate to mate in the surrounding area, potentially increasing health risks for the people there. More information is needed to either confirm or rule out this possibility." (Center for Food Safety 0344 p.13; GeneWatch UK 0335 p. 11) [Footnotes omitted]

2. <u>Possibility that Other Mosquito Species Might Displace *Ae. aegypti*</u>

Several comments (0226, 0329, 0335, 0342, 0344) expressed concern that reduction of *Ae. aegypti* numbers in the test area might result in other mosquito species displacing *Ae. aegypti*.

Anonymous (0329) stated that:

> "GM mosquitoes could alter the population of other mosquito species, such as Aedes albopictus, in an area." (Anonymous 0329 p. 2)

Anonymous (0226), noting that unlike removing breeding sites or using larvicides, Oxitec's single-species approach "does not reduce populations of non-target species", stated that:

> "If population suppression of *Aedes aegypti* is successful (even temporarily), one important question is whether *Aedes albopictus* (Asian tiger) mosquitoes, which also transmit dengue and several other viruses (including chikungunya), will increase in numbers and perhaps establish in new areas as a result of competitive displacement of one species by another. *Aedes albopictus* has been responsible for epidemics of dengue and chikungunya elsewhere in the world and for the reemergence of dengue in southern China, and this species is likely to play an important role in the maintenance and transmission of the virus." (Anonymous 0226 p. 10) [Footnotes omitted]

The Center for Food Safety (0344) also noting that unlike removing breeding sites or using larvicides, Oxitec's single-species approach "does not reduce populations of non-target species" stated that:

> "If population suppression of *Aedes aegypti* is successful, one important question for the risk assessment is whether *Aedes albopictus* (Asian Tiger) mosquitoes, which also transmit dengue and other viruses (including chikungunya), will increase in numbers and perhaps establish in new areas as a result of competitive displacement of one species by another. *Aedes albopictus* is widespread in the USA, including in Texas and Florida." (Center for Food Safety 0344 p. 10) [Footnote omitted]

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "In a draft risk assessment for its OX513A strain from 2011, Oxitec states (page 25): "*It is not clear to what extent Ae. albopictus could or would expand its range into areas currently dominated by Ae. aegypti but it is reasonable to expect a degree of such expansion if no countervailing activities are undertake*n". Oxitec has also published a paper which uses computer modelling to show how *Aedes aegypti* and *Aedes albopictus* may interact." (Center for Food Safety 0344 p.12; GeneWatch UK 0335 p. 10) [Emphasis in the original] [Footnotes omitted]

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "Both species can spread extremely rapidly and can interact with and displace one another: for example, *Aedes albopictus* has replaced *Aedes aegypti* in much of Florida and in Bermuda. The results of a 2013 study show that Florida *Aedes aegypti* and *Aedes albopictus* mosquitoes are both competent vectors of the DENV-1 strain of dengue isolated from Key West in 2010." (Center for Food Safety 0344 p.12; GeneWatch 0335 p. 10) [Emphasis in the original] [Footnotes omitted]

Friends of the Earth (0342) stated that:

> "One potential concern is that if Oxitec's mosquito were to successfully reduce the *Aedes aegypti* population and reduce competition for breeding sites, there could be a new ecological niche for other pests to fill, such as the *Aedes albopictus* (Asian Tiger Mosquito). The Oxitec company in its application for the Cayman Island trials warned of the possibility of needing to control for *Aedes albopictus* as that mosquito might increase in numbers. In the Oxitec trials in Panama, the *Aedes albopictus* population was shown to have increased. The Asian Tiger Mosquito is one of the most invasive species on the planet and research has shown it is a possible vector for dengue fever and other tropical diseases, possible leading to more harm for human health. The Asian Tiger

> Mosquito is widespread in the USA, including in Texas and Florida." (Friends of the Earth 0342 p. 3) [Footnotes omitted]

Friends of the Earth (0342) went on to point out that:

> "Oxitec's intention of elimination targets one vector, whereas other vector control methods target breeding grounds for many vectors, either through removing breeding sites in an area or by using repellents for many species." (Friends of the earth 0342 p. 3)

The Center for Food Safety (0344) stated that:

> "Oxitec is [sic] its proposal to the Cayman Islands for its release there noted that the company might also have to follow up with engineering *A. albopictus* as it was expected that the *albopictus* would move into the niche formerly used by *A. aegypti.* The Panama results were published in 2015 in Pest Management Science. This paper finds that the competitor mosquito species Aedes albopictus was increasing significantly year upon year at each of three study sites (one release and one control site) during the experiments." (Center for Food Safety 0344 p. 2) [Footnote omitted]

Some commenters (0329, 0335, 0342, 0344) offered opinions on potential consequences of *Ae. aegypti* being replaced in the test area by another mosquito species, e.g., by *Ae. albopictus* or by *Culex* spp.

i. Potential consequences if *Ae. aegypti* is replaced by *Ae. albopictus*

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "The authors acknowledge that this could have important consequences for the persistence of disease. In its application to the Cayman Islands, Oxitec states: "*Should Aedes albopictus begin to occupy the Aedes aegypti niche upon reduction in their numbers, a concurrent operation will begin to reduce the numbers of Aedes albopictus*". However, no such operation has ever taken place, so there is no evidence it would be effective or cost-effective. More recently, Oxitec's former Chief Scientific Officer, Luke Alphey stated, "*Since Aedes aegypti and* Aedes albopictus *are known to compete … it is possible that the successful implementation of* …[GE mosquito] *gene drives could lead an existing* Ae. aegypti *population to be displaced by* Ae. albopictus *where it would not otherwise have been. This would likely hamper efforts to eliminate viruses such as dengue since* Ae. albopictus *are also competent vectors*...." (Center for Food Safety 0344 p.12; GeneWatch UK 0335 p. 10) [Emphasis in the original] [Footnotes omitted]

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> *"Aedes albopictus* has been responsible for epidemics of dengue and chikungunya elsewhere in the world and for the re-emergence of dengue in southern China. The role of *Ae. albopictus* may have been underrated and this species is likely to play an important role in the maintenance and transmission of the virus. Oxitec frequently cites a review by Lambrechts et al. (2010) to support its claim that *Ae. albopictus* is a less effective vector of dengue than *Ae. aegypti*. However this paper also warns that it is not possible to predict the epidemiological outcome of competitive displacement of *Ae. aegypti* by *Ae. albopictus* and warns that vector status is a dynamic process that in the future could change in epidemiologically important ways." (Center for Food Safety 0344 p.12-13; GeneWatch UK 0335 p, 10-11) [Emphasis in the original] [Footnotes omitted]

Center for Food Safety (0344) and GeneWatch UK (0335) pointed out that:

> "Grard et al. (2014) have identified the presence of ZIKV (Zika virus) in *Aedes albopictus* in Gabon." (Center for Food Safety 0344 p.13; GeneWatch UK 0335 p. 11) [Footnote omitted]

GeneWatch UK (0335) requested that any assessment specifically include:

> "Confirmation that *Aedes aegypti* is the main vector of zika and that other species do not also play a role." (GeneWatch UK 0335 p. 16)

ii. <u>Potential consequences if a non-*Aedes* mosquito species can affect disease transmission</u>

The Center for Food Safety (0344) stated that:

> "In the case of zika, some scientists have argued that common *Culex* species of mosquitoes may also play an important role in transmission of disease. Although the evidence is not definitive (and some scientists have found that *Culex* species do not appear to transmit zika in some regions) the southern house mosquito, *Culex quinquefasciatus*, also known as the common mosquito, may be a vector for zika in certain environments. If this is the case, attempting to reduce zika transmission by targeting *Aedes aegypti* may be the wrong approach." (Center for Food Safety 0344 p.13) [Footnotes omitted]

C. Comments on Potential for Environmental Releases of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations

Several commenters expressed concern that the use of antibiotics to produce products such as OX5034 could contribute to increases in antibiotic resistance in microbial populations. (0037,

0205, 0221, 0226, 0290, 0293, 0329, 0331, 0334, 0335, 0342, 0344). Some commenters described why an increase in resistance to antibiotics in microbial pathogen populations is a public health concern. Other comments offered hypotheses on how use of tetracycline to produce OX5034 mosquitoes could be a pathway to increases in antibiotic resistance in microbial populations. Other commenters offered explanations of why it is unlikely that use of tetracycline to produce OX5034 would affect antibiotic resistance in microbial pathogen populations. (0341)

Anonymous (0329) pointed out that:

> "Oxitec uses tetracycline in the breeding process . . . ." (Anonymous 0329 p. 1-2)

Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "Oxitec feeds its GE mosquitoes on the antibiotic tetracycline, as this acts as a chemical switch to turn off the genetic killing mechanism." (Center for Food Safety 0344 p. 10; GeneWatch UK 0335 p. 8)

J.W. Norris (0334) explained that:

> "This transgenic mosquito has been bred with a dominant lethal gene that is artificially repressed to allow insects to be reared; this system is called RIDL (Release of Insects carrying a Dominant Lethal). Tetracycline is the agent used to prevent expression of the lethal gene; it is added into the water in which the mosquitoes are bred for this purpose. When these mosquitoes are mass produced, it requires a lot of tetracycline to rear them as well as a lot of human employees to manufacture and distribute them. The fundamental design flaw of the RIDL system is that an antibiotic, when used by itself, will select for resistance." (J.W. Norris 0334 p. 1 of the Attachment) [Footnote omitted]

Friends of the Earth (0342) stated that:

> "Oxitec's mosquitoes are engineered to be dependent on the presence of tetracycline and to die in its absence. In theory, the males will mate and the females die off while their tetracycline-dependent gene passes onto their offspring. The female offspring should die in the late larvae or pupae stage, . . ." (Friends of the Earth 0342 p. 3)

Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "The use of tetracycline to breed the GE mosquitoes in the lab carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health." (Center for Food Safety 0344 p. 10; GeneWatch UK 0335 p. 8)

J.W. Norris (0334) stated that:

> "When these mosquitoes are mass produced, it requires a lot of tetracycline to rear them as well as a lot of human employees to manufacture and distribute them. The fundamental design flaw of the RIDL system is that an antibiotic, when used by itself, will select for resistance." (J.W. Norris 0334 p. 1 of the Attachment)

1. Why Increases in Antibiotic Resistance in Microbial Pathogens is a Concern

Some commenters (0329, 0334) offered reasons for stating that increases in antibiotic resistance in microbial pathogens is a concern.

J. W. Norris (0334) argued that increasing resistance to antibiotics in microbial pathogen populations is a public health problem that must be taken into account in assessing products of antibiotic dependent technologies such as that used to create OX5034. In a petition submitted to the docket, while recognizing the serious threat posed by mosquito borne diseases, he stated that:

> "The primary hesitation after review of the technology stemmed from the use of tetracycline as a necessary cofactor for mosquito development. Antibiotic resistance is another epidemic those in public health must consider in evaluation of this technology. In 2013, the CDC estimated that in the United States alone more than two million people are sickened every year with antibiotic-resistant infections, with at least 23,000 dying as a result. These estimates are based on conservative assumptions and are likely minimum estimates. If the issue of antimicrobial resistance is not addressed by 2050, 10 million people are expected to die annually from resistant organisms. The economic analysis of such a rise in resistance by 2050 has also lead to a predicted reduction of 2% to 3.5% in GDP and cost the world up to 100 trillion USD. … For this reason, we need to ensure that our solution to these mosquito-borne illnesses does not exacerbate the global antimicrobial resistance epidemic." (Norris attachment 0334 p. 1) [Footnotes omitted]

Anonymous (0329) noted the importance of tetracycline in controlling certain microbial pathogens stating that:

> "Tetracycline is used to treat MRSA and its non-medical use may lead to tetracycline resistant MRSA." Anonymous 0329 p. 1-2)

2. Routes Through Which Microbial Populations Might be Exposed to Tetracycline

Commenters raised the possibility that production and use of OX5034 might lead to increased antibiotic resistance in microbial populations, including in microbial pathogens, through two different pathways: through the disposal of tetracycline-containing waste materials from the OX5034 breeding process, or by the release of mosquitoes carrying resistant bacteria.

i. Potential for waste from breeding operations to lead to increased antibiotic resistance in microbial populations

Anonymous (0329) stated that:

> "The waste from this tetracycline has the potential to increase antibiotic resistant pathogens". Anonymous 0329 p. 1-2)

Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "Disposal of waste water, containing tetracyclines and/or tetracycline-resistant bacteria, may also spread antibiotic resistance." (Center for Food Safety 0344 p. 10; GeneWatch UK 0335 p. 8)

ii. Potential for releases of the tetracycline-exposed OX5034 mosquito to lead to increased antibiotic resistance in microbial populations

Several commenters questioned whether OX5034 mosquito's tetracycline-exposed microbiome could be a route to increased antibiotic resistance in microbial populations. One commenter described in detail how exposure to an antibiotic could affect a mosquito's microbiome beyond its use in production of OX5034 males.

Center for Food Safety (0344) and GeneWatch UK (0335) stated that resistant bacteria could be associated with OX5034:

> "A postgraduate student working with Oxitec's GE *Aedes aegypti* mosquitoes has conducted relevant experiments which found that "*Colonies grew on plates supplemented with 50 µg ml-1 of chlortetracycline, indicating that the larvae bore chlortetracycline-resistant bacteria*". (Center for Food Safety 0344 p. 10; GeneWatch UK 0335 p. 8) [Emphasis in the original] [Footnote omitted]

Center for Food Safety (0344), GeneWatch UK (0335) and Anonymous (0226) explained that "the use of tetracycline to breed the GM mosquitoes in the laboratory also carries the risk of spreading antibiotic resistance" because:

> "Insect guts are reservoirs for antibiotic resistance genes with potential for dissemination. Insect production in factories exposed to antibiotics could lead to drug resistance in their microbiota so that the insects disseminate antibiotic resistance when released into the environment." (Center for Food Safety 0344 p. 10; GeneWatch UK 0335 p. 8; Anonymous 0226 p. 11) [Footnotes omitted]

Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "Oxitec's letter to the EPA states that released male OX5034 *Aedes aegypti* will be reared in the absence of tetracycline. This is not possible for the OX513A strain, but is possible for OX5034, because the latter strain is female-killing only (so male larvae do not need to be fed the antibiotic in order to survive). However, the OX5034 strain requires tetracycline at the egg production stage as the female parents of the males intended for release need the antibiotic in order to survive to adulthood to lay their eggs. Thus, **there will likely be tetracycline-resistant bacteria in the egg stage of the GE males, which may persist until their release on adulthood.**" (Center for Food Safety 0344 p. 10; most of this comment repeated by GeneWatch UK 0335 at p.8) [Emphasis in the original]

Center for Food Safety (0344) and GeneWatch UK (0335) added that:

> "**There is also potential for intergenerational transfer of antibiotic resistant bacteria**, although we are not aware of any studies of this in *Aedes aegypti*." (Center for Food Safety 0344 p. 10; most of this comment repeated by GeneWatch UK 0335 at p.8) [Emphasis in the original]

Similarly, Anonymous (0329) stated that:

> "GM mosquitoes exposed to tetracycline during the breeding process may pass antibiotic resistant bacteria to their offspring." Anonymous 0329 p. 1-2)

J.W. Norris (0334) explained that:

> "The fact the OX5034 males do not physically contact tetracycline themselves in no way negates the previous concerns about promotion of antibiotic resistant bacteria and spread via OX5034 males to human environments of these resistant bacteria. The OX5034 breeding females require enough tetracycline to get into every cell of these genetically modified females to shut off the lethal proteins at the implanted genetic switch. Such exposure to an antibiotic will press the microbiome of the females to antibiotic resistant bacteria. When the OX5034 females lay eggs, they will share this pressed microbiome with their eggs. This is much the same way chicken eggs become

> contaminated with salmonella. Salmonella is screened for in chicken eggs, leading to chicken egg recalls. When the OX5034 eggs hatch they will contaminate the larva and the larval trays with these pressed bacteria. If allowed to mature and be released, these OX5034 males will search out wild female and mate with them. The act of mating will contaminate the wild females with the antibiotic pressed bacteria of the parent much like an sexually transmitted disease. When the wild female feeds on a human, there is concern about human contamination with resistant, possibly pathogenic bacteria." (J.W. Norris 0334 p. 1)

J.W. Norris (0037) stated that the dependence of OX5034 breeding females on tetracycline is:

> " . . .10X THE TETRACYCLINE OF VERSION OX513A." (J.W. Norris 0037 p.2)[Emphasis in the original]

J.W. Norris (0037) stated that consistent with the request made for OX513A and similar to what is done with chicken eggs where there is potential for salmonella contamination, for OX5034 :

> " . . . we continue to desire a similar evaluation and supervision process here. Eggs must be proven low human health risk of processed driven large numbers of transferable resistant bacteria." (J.W. Norris 0037 p.2)

With regard to the possibility of pathogenic bacteria being transmitted, J.W. Norris (0334) also stated that:

> "The water in which the mosquitoes are bred is not sterile, nor are the mosquito that are bred in them. Several images provided by news outlets from OX513A production facilities demonstrate hand contact without gloves, an employee placing his ungloved thumb in a larvae-filled container, . . , of greater significance is the seeding of larvae with bacteria from the employee's hand as well as the spread of bacteria from the tetracycline baths onto the employees hand. These tetracycline baths – from handling by human employees to the very fact living organisms are bred in them – have the potential to become major bacterial breeding sites." (Norris 0334 p. 1-2 of the Attachment)

J.W. Norris (0293) added that:

> "The fact that there is a mold issue in production suggests a hygiene issue greater than expected." (J.W. Norris 0293 p. 1)

J.W. Norris (0334) further explained the pathway through which he believes resistance to antibiotics could be increased through releases of OX5034 mosquitoes:

> "The process of pupal washing is not sufficient for the purpose of allaying resistance concerns. Once larva begin to pupate they are removed from the tetracycline baths and rinsed 4 to 6 times in fresh tap water. They are then placed in a tap water bath. This may be sufficient for removing excess tetracycline, but the bacterial film from the tetracycline bath must be expected to remain. The promotion of tetracycline resistant bacteria coating the external surface of each pupae must therefore raise concern the new bath will be contaminated by this bacteria." (J.W. Norris 0334 p. 2 of the Attachment)

J.W. Norris (0334) went on to explain that:

> "The emergence of the adult mosquito from its pupal case provides an additional concern for contamination of the adult. An adult *Aedes aegypti* first emerges from its pupal case by displaying its legs and pushing out of its external pupal casing. (Appendix B) Contamination would be unavoidable in the likely setting of resistant bacteria. Furthermore, Coon et al. showed using PCR that *E. coli* that had colonized axenic larvae was transstadially transmitted to adult *Ae. Aegypti*. The fact molecular tetracycline is likely negligible is irrelevant as the future generation of bacteria do not have a lethal gene and can be expected to genetically have the inheritance of their tetracycline bath forbearers." (J.W. Norris 0334 p. 2 of the Attachment) [Footnote omitted]

Finally, J.W. Norris (0334) went on to explain that OX5034 mosquitoes may be able to transmit tetracycline resistant bacteria to human living environments:

> ". . . , there is also the possibility of spread of the bacteria by the mosquitoes themselves. Recent publication by Junqueira et al demonstrates flying insects can mechanically contaminate an environment with bacteria they receive in a previous environment. This research was focused on the blow fly and the house fly but demonstrates capably the ability for promoted resistant bacteria from an environment such as the tetracycline maturation trays . . . to be transplanted mechanically to target areas such as people's homes . . . ." (J.W. Norris 0334 p. 2 of the Attachment) [Footnote omitted]

J.W. Norris (0334) further explained that:

> "This means the very mechanism that gives the OX513A efficacy in combating native *Aedes aegypti* – the desire for males to seek out females in human habitats - would be the very same delivery system for mechanical contamination of landing sites in and around people's homes. With this in mind, immunocompetence of the release area population should not be ignored. Those who are at greater risk for bacterial infection are routine members of all human communities. These individuals include diabetics,

asthmatics, HIV, COPD, and others for whom antibiotic resistance would present heightened risk." (J.W. Norris 0334 p. 2 of the Attachment)

3. Other Antibiotic Resistance Considerations

GeneWatch UK (0335) and the Center for Food Safety (0344) questioned whether antibiotics in addition to tetracycline (penicillin and streptomycin) were used to produce OX5034 and whether resistance to these antibiotics could also be driven by their use in OX5034 production. They stated that:

> "Protocols described in documents released in response to the GeneWatch UK Freedom of Information requests raise further questions about the use of antibiotics by Oxitec. The documents reveal that the company feeds its adult OX513A *Aedes aegypti* mosquitoes on sugar solution containing the antibiotics penicillin and streptomycin, during egg production (Section 1.2 of the Quality Control Protocol for the Assessment of Mating Competitiveness, page 88 of the pdf; and Section 1.2 of the Quality Control Protocol for Colony Genotyping, page 101 of the pdf). . . . It is unclear from the information provided, whether penicillin and streptomycin are fed to adult GE mosquitoes only during specific experiments, or also during mass production, prior to open release into the environment." (GeneWatch UK 0335 at p. 8-9; Center for Food Safety 0344 p. 10) [Footnote omitted]

GeneWatch UK (0335) and the Center for Food Safety (0344) indicated that knowing when these antibiotics were used was important because:

> "• The scale of the disposal problem would increase if these antibiotics are used during mass production;
>
> • It could lead to the spread of antibiotic resistant bacteria by the GE mosquitoes on release; . . ." (GeneWatch UK 0335 at p. 8-9; Center for Food Safety 0344 p. 10)

4. Could Antibiotic Use Affect the Mosquito's Competency at Vectoring Pathogens

Two commenters expressed concern that antibiotics may affect *Ae. aegypti* competency to vector pathogens. These commenters, Center for Food Safety (0344) and GeneWatch UK (0335), stated that:

> "There is some evidence that antibiotics may increase the transmission of dengue fever by *Aedes aegypti* mosquitoes." (GeneWatch UK 0335 p. 8-9; Center for Food Safety 0344 p. 10) [Footnote omitted]

5. Comments Rebutting Concerns About the Potential for Releases of OX5034 to Increase Antibiotic Resistance in Microbial Populations

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) stated that the "potential for Oxitec's mosquito technology and its subsequent deployment to lead to increased risk of antibiotic resistance, is negligible" because:

> "Oxitec makes use of a small level of tetracycline-family antibiotics in the rearing of our 2nd generation mosquito eggs in its facility in the UK. Oxitec technology does not increase risk of antibiotic resistant bacteria in the environment where egg manufacture or releases are carried out (as confirmed by the FDA in 2016), and Oxitec will not be using any tetracycline or any other antibiotic in the US." (N. Rose 0341 p. 8)

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) furthering the argument that the "potential for Oxitec's mosquito technology and its subsequent deployment to lead to increased risk of antibiotic resistance, is negligible," added that:

> **"• No tetracycline or other antibiotics will be used in rearing of Oxitec's non-biting male mosquitoes for the pilot project in the Florida Keys**; **no tetracycline will be released into the environment in the US; Oxitec will have no tetracycline in the US. . . .**
>
> "**• The Oxitec male OX5034 mosquitoes reared for release in Florida will never have been in contact with tetracycline, and therefore the risk of spreading tetracycline-resistant bacteria is negligible."** (N. Rose 0341 p. 8) [Emphasis in the original]

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) explained that:

> "• Oxitec will use only a small level of doxycycline, a common, widely-used member of the tetracycline family, only in the UK, to rear females which will not be released (but which produce the mosquito eggs to be used in Florida).
>
> "• The amount of doxycycline that would be used in the UK to produce the females that would supply all the eggs needed for the EUP is less than 5 grams." (N. Rose 0341 p. 8)

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) arguing that "existing human and agricultural uses of tetracyclines are far more likely to result in the spread of antibiotic-resistant bacteria, than Oxitec's very limited use of doxycycline outside of the USA" stated that the amount of antibiotic Oxitec would use:

". . . is about the equivalent of two 10-day courses of antibiotics to treat a normal infection. More than 6.5 million courses of doxycycline were prescribed in the US in 2016.

• In addition, the EPA, federal and Florida state governments have approved the deployment of hundreds of tons of antibiotics into Florida's environment annually for agricultural and food production purposes.

• Agricultural use of antibiotics in Florida alone is 88 million times more than what Oxitec will use in the UK." (N. Rose 0341 p. 8) [Footnotes omitted]

N. Rose, Head of Regulatory Science at Oxitec Ltd., (0341) pointed out that:

"When the FDA approved Oxitec's 1st generation technology in 2016, it considered Oxitec's use of antibiotics and determined that there is no risk to humans, animals or the environment from use in Oxitec's rearing processes in the US. Now Oxitec's 2nd generation technology does not use any tetracycline/doxycycline in the US for rearing of male mosquitoes for release into the environment." (N. Rose 0341 p. 8)

D. Comments Concerning Other Means By Which OX5034 Might Present Risk to the Environment

A few commenters (0132, 0147) argued that there are other means by which OX5034 might present risk to the environment.

Commenter A. Johnson (0132) objected to OX5034 because:

" . . . can be seen in Brazil as a massive failure bc the GMO mosquitoes bred with native ones and now are just as bad if not worse.... But that's almost EXACTLY what happened back in the 50s with the Africanized honey bees! Quit screwing with insects bc they're too small and rapid breeders to contain or reign in once out of control! It is 2019 and Africanized honey bees are still deadly, still invasive species, and still a major problem. And they're bigger than mosquitoes!" (A. Johnson 0132 p. 1)

Anonymous (0147) stated that:

"I live in an area that has to deal with a similar "experiment" gone wrong. Killer bees took a while to travel up to the southwest from South America where the queen bee was accidentally released. This genetic experiment will be an intentionally created

disaster. In other words, this is an attempt to fix a problem created by technology with technology." (Anonymous 0147 p. 1)

EPA Responses to Comments in Unit VIII.A. – Effects on Food Supply. With regard to the comments expressing concern that the ecosystem would react dynamically when large numbers of male *Ae. aegypti* mosquitoes are released into it, e.g., affecting the ability of animals in that system to find food, the Agency examined this question in detail in the Human Health and Environmental Risk Assessment found in the docket established for this action. The document concluded that testing under the EUP would have no adverse effects on organisms specifically mentioned in the comments, i.e., on birds, dragonflies, bats, amphibians (frogs) or lizards. It is highly unlikely that any of these species would be reliant on *Ae. aegypti* because, as a non-native species, the mosquito has not been present in the North American ecosystem for sufficient time to develop an essential ecosystem function. Relevant findings from Unit II.D.2, "Ecological exposure and risk characterization," of the Human Health and Environmental Risk Assessment located in the docket established for this action can be found below:

> With regard to birds, several types of birds including most varieties of swallows, warblers and other songbirds consume mosquitoes among other flying insects. However, the mosquito is likely to form only a small part of the bird diet. Perhaps the most frequently anecdotally cited bird as a consumer of mosquitoes is the Purple Martin (*Progne subis*), the largest species of martin in North America. However, reports of foraging studies have not found that mosquitoes constitute a significant portion of the Purple Martin diet[6] and instead mosquitoes typically do not make up more than 3% of the Purple Martin diet[7].
>
> With regard to dragonflies, dragonflies are known to eat adult mosquitoes; however, they also consume butterflies, moths and smaller dragonflies which serve as significant energy sources, thus mosquitoes are likely not an essential part of their diet.
>
> In terms of lizards and frogs, lizards and frogs are vertebrates and while it is not known that any vertebrates have evolved to specifically target *Ae. aegypti* mosquitoes as a major portion of their diet, in some instances, mosquitoes can constitute a source of prey. Mosquitoes do not form a large part of the lizard diet, although these reptiles may consume mosquitoes they capture opportunistically. Frogs, tadpoles and toads are amphibians and can all eat mosquitoes, but these organisms do not rely on mosquitoes for a substantial part of their diet. Given the limited time during which OX5034 testing is

[6] Wiggens, D. A. 2005. Purple Marting (*Progne subis*): a technical conservation assessment. Access date: April 27, 2020 http://www.fs.fed.us/r2/projects/scp/assessments/

[7] American Mosquito Control Association. Do Purple Martings help reduce mosquitoes? Access date: April 27, 2020 https://www.mosquito.org/page/FAQ?&hhsearchterms=%22fan%22#Do%20Purple%20Martins%20help%20reduce%20mosquitoes?

to occur and given that the area of testing is human habitat, it is unlikely that OX5034 releases would adversely affect reptile or amphibian populations.

With regard to pollination, *Ae. aegypti* is not known to have any direct interaction with pollinators, nor to be an effective pollinator itself; thus testing of OX5034 is not expected to adversely impact pollinators or plant populations.

With regard to bats, insectivorous bats are often anecdotally regarded to be a significant predator of mosquitoes and are thought to eat large quantities of mosquitoes. However, in areas where larger, more nutritious insect prey are available, bats do not consume large numbers of mosquitoes as they do not constitute significant calories or nutrients relative to the task of predating upon them. Bats therefore are rarely if ever reliant on *Ae. aegypti* mosquito populations as a sole food source, and the limited OX5034 numbers involved in the EUP testing indicate that the mosquito releases associated with the test will have no effect on bats.

With regard to the comments describing potential fluctuations in mosquito population densities that could occur during testing that may in turn impact the food supply, the findings discussed above can also be applied here. Possible fluctuations in mosquito population densities do not alter the conclusion that *Ae. aegypti* does not serve as a sole or critical food source. Additionally, *Ae. aegypti* is but one species of mosquito, and there are numerous other mosquito species present in the test locations that may represent prey to the various organisms mentioned.

EPA Responses to Comments in Unit VIII.B.1. – Population Dynamics; Potential for Wild Mosquitoes to Move to Surrounding Areas. With regard to comments describing potential fluctuations in mosquito population densities in areas surrounding the test area caused by migration from the test site to the surrounding area, the modeling study cited by the commenters (Yakob et al. 2008)[8] found that although migration into untreated areas may occur in sterile insect releases, this effect was not seen in simulations of releases of insects carrying a dominant lethal gene (RIDL), of which the OX5034 mosquito is one. The commenters did not provide information regarding the other two studies cited (Carvalho et al. 2015 or Harris et al. 2012)[9],[10] to indicate that any fluctuations seen in mosquito density in untreated areas was

[8] Yakob L, Alphey L, Bonsall MB (2008) *Aedes aegypti* control: the concomitant role of competition, space and transgenic technologies. *Journal of Applied Ecology* **45**(4):1258–1265.

[9] Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, … Capurro ML (2015) Suppression of a Field Population of *Aedes aegypti* in Brazil by Sustained Release

[10] Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, Oviedo MN, Lacroix R, Naish N, Morrison NI, Collado A, Stevenson J, Scaife S, Dafa'alla T, Fu G, Phillips C, Miles A, Raduan N, Kelly N, Beech C, Donnelly CA, Petrie WD, Alphey L (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat. Biotech*., **30**(9), 828–830.

significantly different from normal mosquito density fluctuations. Therefore, because the commenters did not provide sufficient information in the context of this EUP request, EPA is unable to respond to the comment.

EPA Responses to Comments in Unit VIII.B.2. – Population Dynamics; Possibility that Other Mosquito Species Might Displace *Ae. aegypti*. With regard to the potential for other non-*Aedes* species to fill any niche vacated by *Ae. aegypti* in the test area, *Ae. aegypti* is an invasive species in the United States so it either replaced another species or occupied an unoccupied niche when it entered the United States. Any changes in abundance of competitor species during OX5034 trials are therefore likely the result of natural ecosystem processes that would occur with reduction in the numbers of the invasive species. However, in the case of *Ae. aegypti*, due to its strong anthropophilic behavior and use of artificial breeding containers, there is little evidence of it displacing native species. Additionally, the local wild *Ae. aegypti* population would be expected to recover to pre-trial numbers after the cessation of OX5034 mosquito releases, and, should there be any changes in mosquito species populations, these would be expected to be short-term.

With regard to the possibility that *Ae. albopictus* might replace *Ae. aegypti* in the test area as a result of the EUP testing, due to numerous successful invasions by both *Ae. aegypti* and *Ae. albopictus*, the current worldwide distribution of these species overlap. As stated by some of the commenters (0344, 0335), both *Ae. aegypti* and *Ae. albopictus* can spread rapidly and are known to displace one another. The commenters did not provide information as to how EUP testing would significantly change these natural species dynamics, nor how any fluctuations in the proportion of either species would result in different risks, as both species are known to vector the same diseases.

EPA Response to Unit VIII.C. – Comments on Potential for Environmental Releases of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations. EPA recognizes the importance of antibiotics in medical and other applications and that increasing resistance to antibiotics in microbial pathogen populations is a public health problem. The Agency considered whether release of OX5034 mosquitoes in the environment could lead to spread of antibiotic resistant bacteria in the Human Health and Environmental Risk Assessment that can be found in the docket established for this action.

Due to the use of antibiotics in the process of producing OX5034 eggs, the presence of antibiotic resistant bacteria in the mosquito microbiome of the OX5034 colony is possible; however, EPA has concluded that there is negligible risk that testing of OX5034 mosquitoes would spread antibiotic resistant bacteria in the US environment for the following reasons.

Although tetracycline analogues will be used in the overall manufacturing of OX5034 (e.g., colony maintenance in the UK), no tetracyclines will be used in the US facilities producing OX5034 male adults for release in the United States, nor will tetracyclines be used in the

release devices for field deployment of OX5034 mosquito eggs. These US conditions eliminate any selective pressure to evolve antibiotic resistance in bacteria in the OX5034 mosquito microbiome or to maintain any that might have been on OX5034 in the UK and thus could exist on eggs shipped from the UK. While OX5034 eggs shipped to the United States could have some bacteria on their surface, the number of resistant bacteria likely to be present are expected to be significantly reduced by the behavior of the mosquito in relation to a microbiome and the conditions of use in the United States. *Aedes* species mosquitoes primarily acquire their gut microbiota from their environment as larvae, and neither the egg release devices nor the facility for producing adult OX5034 will use tetracyclines, making the presence of antibiotic resistant bacteria unlikely. Additionally, conditions in the rearing box are also designed to minimize bacterial growth, further reducing the likelihood of any substantial amount of antibiotic resistant bacteria being present.

Therefore, due to the lack of antibiotics used in the United States in egg release devices or in the production of OX5034 male mosquitoes for release, coupled with the fact that mosquitoes generally acquire their microbiome from their environment, the probability that releases of OX5034 male mosquitoes during the testing would spread antibiotic resistant bacteria in the environment is very low.

Similarly, because no tetracyclines will be used in the US facilities producing OX5034 male adults for release in the United States, nor will tetracyclines be used in the release devices for field deployment of OX5034 mosquito eggs, the question of whether disposal of wastewater could spread antibiotic resistance does not apply.

With regard to the comment offering the example of bees as insects that transstadially transfer their microbiota, bees, in contrast to mosquitoes, have specialized microbiota that are maintained by direct transfer between individuals.

With regard to the comment that OX5034 males through mating could potentially contaminate wild females with any bacteria present on OX5034, research suggests that bacteria that form a symbiosis with the mosquito early in development inhibit colonization by other bacteria. As noted above, the probability that OX5034 males microbiome would contain tetracycline resistant bacteria is very low due to the conditions of production in the United States, and this consideration in addition to the resident microbiomes' ability to protect against contamination suggest that the probability that local wild females could be contaminated with antibiotic resistant bacteria by OX5034 males is negligible.

With regard to the comment that male OX5034 mosquitoes might transfer antibiotic resistant bacteria from growth trays to homes, tetracycline containing growth trays were used for the OX513A mosquito but as noted above, tetracycline will not be used in the US facilities or rearing boxes of the OX5034 mosquito, thereby eliminating such risk.

With regard to the comment that antibiotics in addition to tetracycline and its derivatives might be used in the production of OX5034, the responses above for comments on use of tetracycline apply equally to any other antibiotics that might be used in producing OX5034.

With regard to the comment expressing concern that antibiotics may affect *Ae. aegypti* competency to vector pathogens, recent studies suggest that the *Ae. aegypti* microbiome can modulate the mosquito immune system and influence vector competence. For example, the removal of the majority of midgut bacteria through antibiotic treatment can result in greater *Ae. aegypti* susceptibility to dengue virus infection. EPA carefully considered the possibility that treatment with antibiotics during colony production could affect vector competency. However, EPA does not find that this consideration affects its analysis because (1) only OX5034 males will form part of the testing, (2) the conditions of production and use of OX5034 in the United States do not select for the maintenance or presence of antibiotic resistant bacteria, and (3) the OX5034 hemizygous offspring resulting from matings between OX5034 males and local *Ae. aegypti* females should have similar microbiomes as offspring from wild mosquitoes because mosquitoes primarily acquire their gut microbiota from their environment as larvae and both OX5034 hemizygous offspring and wild mosquito larvae will develop in the natural environment (i.e., be exposed to similar microbes).

EPA Response to Unit VIII.D. – Comments Concerning Other Means By Which OX5034 Might Present Risk to the Environment. With regard to comments comparing releases of OX5034 mosquito under the controlled conditions of an EUP to the incident resulting in the establishment of Africanized bees in the American continents, the Africanized bee was first introduced to Brazil in 1956 in an effort to increase honey production. However, several swarms escaped quarantine in 1957. Since then the Africanized bees have spread through South America and entered North America in 1985. Unlike the Africanized bees that display several behavioral characteristics that aid invasive behavior, OX5034 is intended to be self-limiting, i.e., to eventually disappear post-release of OX5034 males, from the wild local mosquito population because of the negative selection pressure exerted by the tTAV-OX5034 gene.

IX. Ability of OX5034 to Produce the Desired Result Including Comments on Efficacy Testing

A number of comments were received on the issue of whether OX5034 would be effective in suppressing mosquito populations. Thirty-one commenters questioned whether OX5034 would be efficacious in the field. (0005, 0014, 0030, 0038, 0047, 0053, 0056, 0059, 0071, 0089, 0095, 0096, 0111, 0114, 0119, 0146, 0154, 0176, 0198, 0219, 0223, 0225, 0226, 0233, 0245, 0259, 0262, 0273, 0277, 0278, 0293, 0306, 0318, 0329, 0331, 0332, 0335, 0342, 0344). Some of these commenters argued that the purported successes of OX513A in venues where testing occurred was contradicted by subsequent reports on those trials. Some commenters argued that OX5034 would not be a sustainable technology. (0046, 0047, 0053, 0056, 0059, 0071, 0089, 0095, 0096,

0111, 0114, 0119, 0146, 0225, 0233, 0245, 0259, 0262, 0273, 0277). For example, commenter A. Hart (0114) stated that releasing "GM mosquitoes is not a sustainable technology, since if the releases are stopped, the populations rebound to pre-release levels." Other commenters questioned the relationship between OX5034 and more traditional control methods (adulticides and larvicides) (0335, 0344), while others suggested OX5034 should be evaluated in comparison to other means of mosquito control. (0030, 0154, 0176, 0219, 0223, 0329, 0331, 0342).

Twenty-three commenters supported issuance of the EUP, with most of these commenters of the opinion that OX5034 would be efficacious in the field or at a minimum should be given the opportunity to show whether it is efficacious in the field. (0024, 0068, 0075, 0122, 0150, 0163, 0191, 0202, 0207, 0212, 0251, 0263, 0276, 0299, 0301, 0321, 0325, 0336, 0338, 0339, 0340, 0341, 0343).

A. Comments Questioning Whether OX5034 Would Be Efficacious

Most of these commenters argued that OX5034 was not efficacious basing their concern on arguments that OX513A had not been proved efficacious. These commenters based their arguments on: (1) reports in the scientific literature analyzing or commenting on data generated during testing of OX513A as well as comments from officials of countries where testing had been conducted; (2) observations that most countries where testing occurred did not continue to use OX513A and did not engage in testing of OX5034; and (3) comments that OX5034 would not be a sustainable technology.

A number of commenters, however, argued for testing of OX5034 to determine efficacy. These commenters expressed the belief that based on previous testing of OX513A and what is known of the RIDL technology, OX5034 will be efficacious for suppressing *Ae. aegypti* mosquito populations.

1. Comments Arguing that OX5034 May Not Be Efficacious Based on Reports in the Scientific Literature or Comments from Officials of Countries Where Testing had been Conducted on OX513A

Several commenters (0198, 0306, 0332, 0335, 0344) argued that OX5034 may not be efficacious in suppressing *Ae. aegypti* mosquito populations. The Center for Food Safety (0344) and GeneWatch UK (0335) referred to previous testing with OX513A to argue that OX5034, built on the same RIDL technology as OX513A, may not be successful in suppressing *Ae. aegypti* mosquito populations.

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that Oxitec's claims that its experiments have been successful:

> ". . . are not supported by the evidence. For example, emails released as a result of Freedom of Information requests to the Cayman Islands' Mosquito Research and Control Unit (MRCU) reveal comments from scientists there with access to the data, which state, "*Whilst Oxitec and MRCU are making public statements proclaiming major reductions in the Aedes aegypti population in the treatment area the data I have seen does not support this.*" and "*To date all the measures recorded have shown no significant reduction in the abundance of Aedes aegypti in the release area.*"(GeneWatch UK 0335 p. 12-13; Center for Food Safety 0344 p. 15) [Emphasis in the original] [Footnotes omitted]

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "Oxitec has conducted experimental open releases of its OX513A GE mosquitoes in the Cayman Islands, Malaysia, Brazil and Panama. In 2018, the Environmental Health Minister in the Cayman Islands confirmed that trials of Oxitec's GE mosquitoes there did not work and would be abandoned." (GeneWatch UK 0335 p. 12; Center for Food Safety 0344 p. 13-14) [Footnote omitted]

The Center for Food Safety (0344) and GeneWatch (0335) stated that:

> "Oxitec's letter to the EPA claims that effective mosquito control has been demonstrated with OX513A, but this contradicts the evidence outlined in the Cayman Islands, Panama and Brazil studies . . . . Further, it claims that effective mosquito control has also been shown for OX5034 in a trial in Brazil: however, there is no published evidence demonstrating this." (Center for Food Safety 0344 p. 15; GeneWatch 0335 p. 13)

The Florida Keys Environmental Coalition (0331), arguing that the numbers posted for suppression in the trials in the Caymans and Brazil remain in strong dispute, stated that:

> "In the Cayman to the chagrin and protest of Dr Wheeler and his staff, the government published a 62% suppression level after over a year of the trial, yet Oxitec posted 96% on their website to this very day. In Brazil the estimate from Danillo Carvalho, Univ of Sao Paulo, suggest suppression in the range of 60 – 70%, but Oxitec claims 92%." (Florida Keys Environmental Coalition 0331 p. 2)

Anonymous (0198) stated that:

> "Note that the so called success presented years ago by Oxitec in Brazil (Carvalho et al. PLoS NTD 2015) has been discussed via a re-analysis of their data in a peer-reviewed

paper published in Lancet Global Health (Boete & Reeves, 2016)." (Anonymous 0198 p.1)

L.M. Castro (0332) stated that:

> "A retrospective review of the environmental impact assessment that Oxitec presented to Brazilian authorities reveals that . . . the outcome of the experimental release of mosquitoes did not achieve the desired goals." (L.M. Castro 0332 p. 2)

2. Observations that Most Countries Where Testing Occurred Did Not Continue to Use OX513A

Some commenters (0306, 0335, 0344) voiced that OX5034 would not be efficacious in suppressing *Aedes aegypti* mosquito populations. The Center for Food Safety (0344) and GeneWatch UK (0335) referred to discontinuation of previous testing with OX513A to argue that OX5034, built on the same RIDL technology as OX513A, to argue that:

> "Oxitec's releases of GE mosquitoes in Panama and Malaysia ceased earlier, due to concerns about costs, effectiveness and risks. In Malaysia, trials were abandoned following a small open release experiment to measure flying distances and survival rates. The health ministry concluded that "*the method was not practical besides involving high costs*". In Panama, open release trials of Oxitec's GE mosquitoes were conducted in 2012 and then ceased, reportedly due to the high costs. Proposed trials in other countries never actually took place. Oxitec notes that its former subsidiaries in Singapore, Mexico, Australia and Costa Rica are all now dormant. Since its Cayman Island operations have now closed, only the company's Brazilian office remains active. In Brazil, Oxitec released GE mosquitoes in Jacobina and Juazeiro in the state of Bahia, from 2011 to 2013. In 2016, Oxitec began larger-scale trials of its GE mosquitoes in Piracicaba, a city located in the state of Sao Paulo. However, in 2018, Oxitec Brazil decided to close its GE mosquito factory in Piracicaba. According to the company, the reason was the transition to the newer OX5034 version of its GE mosquitoes, which began to be released in a pilot project in Indaiatuba in the Campinas region, in mid-2018. In November 2018, Oxitec announced that in future it would only conduct trials with this new generation of GE insects." (GeneWatch UK 0335 p. 12; Center for Food Safety 0344 p. 13-14) [Emphasis in the original] [Footnotes omitted]

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "Oxitec's decision to stop releasing its OX513A mosquito and begin trials with a new female-killing version effectively confirms that its trials to date have all been a failure. In Brazil, commercial releases have never been approved by the Brazilian health authority ANVISA, which wants to see evidence of benefits to health before giving its approval, in

line with recommendations from the World Health Organisation (WHO). There is no commercial approval for releases because the company lacks any evidence of efficacy in tackling dengue or other diseases spread by this mosquito." (GeneWatch UK 0335 p. 13; Center for Food Safety 0344 p. 15) [Footnotes omitted]

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "Further, GE mosquito production is extremely costly and there have been production problems. In 2014, the release of 300,000 GE mosquitoes in Panama was reported to have cost $620,000 (more than $2 per mosquito). In the Cayman Islands, production issues included the release of a high percentage of female GE mosquitoes, high adult and larval mortality, and mould in the rearing unit." (GeneWatch UK 0335 p. 13; Center for Food Safety 0344 p. 15) [Footnotes omitted]

3. <u>Comments Expressing the Opinion that OX5034 Would Not be a Sustainable Technology</u>

Some commenters (0046, 0047, 0053, 0056, 0059, 0071, 0089, 0095, 0096, 0111, 0114, 0119, 0146, 0154, 0225, 0226, 0233, 0245, 0259, 0262, 0273, 0277, 0278, 0293, 0306, 0318, 0329, 0331, 0332, 0342, 0344) thought that OX5034 would not prove to be a sustainable technology.

L.M. Castro (0332) stated that:

> "A study by Garziera et al concluded that "the effectiveness of the release program began to break down after about 18 months, i.e., the population which had been greatly suppressed rebounded to nearly pre-release levels." (L.M. Castro 0332 p. 2) [Footnote omitted]

Referring to the Evans et al paper, Anonymous (0225) stated that:

> "Based on previous experience with a release of another variety of Oxitec GM mosquitoes in Brazil, Oxitec's claims that its GM mosquitoes are self-limiting and will result in a sustainable decrease in the wild population are not reliable. According to a peer reviewed Yale study, the GM mosquitoes released in Brazil reproduced and their GM genes contaminated the wild mosquito population. The Yale study also found that the release in Brazil did not result in a sustainable decrease in the mosquito population." (Anonymous 0225 p. 1)

B. Potential Effect of Integrated Pest Management on OX5034 Efficiency

Some commenters (0335, 0344) were concerned about the relationship between OX5034 and more traditional control methods (adulticides and larvicides). GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "The role of Oxitec's GE mosquitoes in Integrated Pest Management (IPM) is also highly questionable. Continuing to use traditional control methods for mosquitoes (adulticides and larvicides) could further limit the effectiveness of Oxitec's technology by killing the GE males before they mate with the wild female mosquitoes, or the larvae before they survive to reproduce the trait and spread it through the wild population. Moreover, since there is little data regarding the effectiveness of existing measures, it is hard to see how the claimed benefits of adding GE mosquito releases to existing measures will be evaluated. On the other hand, failure to use existing control methods (if and when they are effective) in order to allow GE mosquito releases to take place, may put people at unnecessary risk of dengue or other diseases, or simply add to the nuisance of mosquito bites, perhaps with negative impacts on tourism or quality of life." (GeneWatch UK 0335 p. 13; Center for Food Safety 0344 p. 15)

C. Comments Arguing For Alternative Approaches

Some commenters (0030, 0154, 0176, 0219, 0223, 0329, 0331, 0342) argued that alternative approaches to *Ae. aegypti* mosquito control are preferable to the use of OX5034. These commenters argued for the use of (1) traditional SIT, (2) Wolbachia infected mosquitoes, and (3) traditional chemical pesticide techniques.

Commenter E. Young (0030) stated that:

> "I don't think GM mosquitoes will be effective - just release enough sterilized males (that don't bite) to compete with wild type for females. It worked with the screw fly and will work with mosquitoes. No need to introduce new genes." (E. Young 0030 p. 1)

T. Ritchie (0223) stated that:

> "We need to do better than this, like China has with irradiating mosquitoes. Why can't we try this method first?
> https://www.the-scientist.com/news-opinion/combination-strategy-nearly-eliminates-invasive-mosquitoes-in-field-66165
> Let's try this instead, please? https://mosquitomate.com/?v=3.0" (T. Ritchie 0223 p. 2)

Commenter K. Later (0154) stated that:

> "There are methods that are more effective and safer for the environment." (K. Later 0154 p. 1)

Anonymous (0176) stated that:

> "GE mosquitoes will harm public health and the local environments and economies where they would be released. Fortunately, there are less costly alternatives to addressing mosquito-borne diseases with far fewer risks." (Anonymous 0176 p. 1)

The Florida Keys Environmental Coalition (0331) argued that if an emergency does arise:

> " . . . Wolbachia infected male releases, represent a more mature, lower concern Sterile Insect Technique (SIT) product with more effectivity, availability and greater product depth that would also be able to prevent any potential back filling *of Aedes albopictus*, which was clearly demonstrated in the Panama trials by Oxitec." (Flroida [sic] Keys Environmental Coalition 0331 p. 3)

Anonymous (0329) stated that:

> "GM mosquitoes are riskier than current control methods." (Anonymous 0329 p. 1)

Arguing that other control methods might be preferable, Friends of the Earth (0342) pointed out that:

> "Oxitec's intention of elimination targets one vector, whereas other vector control methods target breeding grounds for many vectors, either through removing breeding sites in an area or by using repellents for many species." (Friends of the Earth 0342 p. 3)

Anonymous (0219) suggested that:

> "Killing the carrier will not get rid of the viruses. They will find a different mosquito or a tick or some other way to spread and survive. And now we're left with a GM mosquito with no natural predators that may be even more dangerous than the one that was eliminated. Kill the virus where it breeds and not the carrier." (Anonymous 0219 p. 1)

D. Comments Arguing That OX5034 is Likely Efficacious

Two commenters (0068, 0341) argued that OX5034 is likely to be efficacious.

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) stated that:

> "The 2nd generation mosquito has been successfully tested in Brazil. In partnership with the municipal vector control authorities in the city of Indaiatuba, the pilot project demonstrated the new strain's effectiveness in suppressing populations of the *Aedes aegypti* mosquito – the primary vector of dengue, Zika, chikungunya and yellow fever – in four densely populated urban communities across the city. Post-trial monitoring has also confirmed that the self-limiting gene does indeed decline and disappear post-release." (N. Rose 0341 p. 1)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) added that:

> "The trial was designed to test a number of performance features of the 2nd Generation OX5034 *Aedes aegypti*, including the performance outcomes generated by the use of two different mosquito release rate levels in dense urban environments. Abundance of wild *Aedes aegypti* was monitored before and during the release program to allow for an accurate evaluation of the trial's impact. Wild *Aedes aegypti* numbers were kept at low levels throughout the high season in all treated neighborhoods, whereas populations in areas untreated by Oxitec's OX5034 *Aedes aegypti* rose as normal." (N. Rose 0341 p. 2)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) further stated that:

> "Relative to the untreated control area, releases of OX5034 male mosquitoes achieved an average of 89% peak suppression across two communities treated with a low release rate of mosquitoes and an average of 93% across two communities treated with a higher release rate. The optimal suppression observed was in one community wherein a 96% peak suppression with the high release rate over a four-week period was achieved. ("Peak suppression" is measured using the highest sustained suppression over a four-week period in an Oxitec-treated site when compared to a control site untreated by Oxitec mosquitoes for the same period of time. This measures the intervention's sustained suppression effect over time, which is a more accurate measure than selecting suppression results from a single day or week.)" (N. Rose 0341 p. 2)

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341), referring to a paper by Garziera et al referenced in the Evans et al paper to suggest that the effectiveness of the OX513A release program began to break down after 18 months, stated that:

> "In fact, (Garziera et al., 2017) states that mosquito populations in the two treated areas remained suppressed for some time after OX513A released ceased: "*The mosquito population in Juazeiro (Mandacaru) remained suppressed for 17 weeks after the release interruption, whereas in Jacobina (Pedra Branca) suppression lasted 32 weeks*." 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." (N. Rose 0341 p. 5) [Emphasis in the original]

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) pointing to the Evans et al paper stated that:

"The paper reports that OX513A releases successfully reduced the wild mosquito population, as the mosquito was designed to do." (N. Rose 0341 p. 3)

Commenter P.L. Goodman (0068), noting that "two of the three SIT techniques are very labor intensive and are probably expensive in their application since they typically involve releasing adult male mosquitoes", stated that:

"Based on the application provided by Oxitec for their second generation genetically engineered solution, the ability to release from eggs, not adults and the multiplier affect which significantly reduces the number of releases, may offer significant improvements here." (P.L. Goodman 0068 p. 2)

EPA Responses to Comments in Unit IX.A.1. and IX.A.2. – Comments Questioning Whether OX5034 Would Be Efficacious; Comments Arguing OX5034 May Not Be Efficacious and Observations that Most Countries Did Not Continue to Use OX513A After Testing. With regard to the comments arguing that OX5034 is not likely to be efficacious based on reports in the scientific literature or comments from officials of countries where testing had been conducted on OX513A, while methods and results developed in countries other than the United States may be helpful for developing testing protocols, and thus informing the necessary parameters for environmental testing, the results of those studies are not relevant to the Agency's decision to issue an Experimental Use Permit under FIFRA section 5. EPA may consider foreign field data at the time of a Section 3 application to complete efficacy evaluation to support the final registration decision. EPA does not rely on opinions or reports in the press in forming its decision concerning the issuance of an Experimental Use Permit, rather the Agency relies on data and peer reviewed information in the literature.

With regard to the comment that GE mosquito production is extremely costly, EPA does not consider this type of information in making a determination as to whether issuance of the EUP would be allowed under the no unreasonable adverse effects standard established by FIFRA. The monetary feasibility and cost-benefit decisions of utilizing a pesticide is decided by the market. While EPA does conduct cost-benefit analyses, these are most often done for pesticides that are already on the market or have a set price point.

With regard to the comments referencing published articles to support the contention that OX513A was not efficacious and thus OX5034 will not be efficacious, EPA evaluates the efficacy

of each product individually and therefore whether other Oxitec, Ltd., products like, OX513A, are efficacious or not is irrelevant to EPA's decision to issue this EUP.

EPA Responses to Comments in Unit IX.A.3. – Comments Questioning Whether OX5034 Would Be Efficacious; Opinions Arguing That OX5034 Would Not Be A Sustainable Technology. With regard to comments that OX5034 would not represent a sustainable technology, these comments appear to be internally contradictory. On the one hand, commenters express concern that the OX5034 mosquitoes might become a permanent part of the wild local mosquito population, while on the other hand, commenters argue that failure to do so indicates a failure of the technology. OX5034 is intended to be self-limiting, i.e., to eventually disappear post-release of OX5034 from the wild local mosquito population because of the negative selection pressure exerted by the tTAV-OX5034 gene. Post release monitoring under the EUP will reveal when the gene for tTAV is eliminated from the mosquito population following cessation of OX5034 adult male mosquito and egg releases. It is also assumed that when releases of OX5034 cease, wild *Ae. aegypti* mosquito populations would then rebound in the test area.

EPA Responses to Comments in Unit IX.B. — Potential Effect of Integrated Pest Management on OX5034 Efficacy. With regard to comments concerning the relationship between OX5034 and more traditional control methods (adulticides and larvicides) during the trial, if such control measures are employed during the OX5034 testing protocol, areas treated with OX5034 mosquitoes and untreated control areas will receive the same treatments to control for the effect of the more traditional mosquito control regimens in OX5034 treated and OX5034 untreated control areas. The registrant will report details of any mosquito abatement programs back to EPA if and when the data from the EUP is submitted for subsequent registration packages.

EPA Responses to Comments in Unit IX.C. — Comments Arguing for Alternative Approaches. With regard to comments expressing a preference for alternative mosquito control techniques, EPA recognizes the value of using multiple techniques in attempting to control *Ae. aegypti* and other mosquito species. EPA has registered multiple traditional adulticide and larvicides, as well as Wolbachia-infected mosquitoes. If successful at suppressing *Ae. aegypti* mosquito populations and registered by the Agency for that purpose, OX5034 would be another tool to utilize to control this mosquito. Market dynamics and consumer choice will drive the free market to choose amongst the available registered tools for mosquito control.

With regard to the comment that other alternative mosquito control products may be preferable because they may be safer, in order to issue an EUP, EPA must determine that there are no unreasonable adverse effects to the environment associated with use of the pesticide for the purposes of gathering data on the pesticide. As part of the Agency's decision to grant this EUP, EPA has found there to be no unreasonable adverse effects associated with release of the OX5034 mosquito under the conditions of the EUP for the purpose of gathering efficacy

data at the proposed locations. EPA has carefully evaluated the data submitted by the registrant to make a no unreasonable adverse effects decision in the Human Health and Environmental Risk Assessment. This document can be found in the docket (EPA-HQ-OPP-2019-0274) established for this action.

With regard to the comment that EPA should kill the virus where it breeds and not the carrier, EPA notes that killing the mosquito, or suppressing its numbers, is in effect killing the virus where it breeds for at least part of its life cycle. Viruses such as dengue, chikungunya or Zika are spread through the bite of the female *Ae. aegypti* mosquito. The female *Ae. aegypti* mosquito becomes infected with the virus when it sucks the blood of a human infected by the virus. Subsequent to entering the female mosquito, the virus multiplies and spreads through the body of the mosquito into the saliva. After about a week, the female *Ae. aegypti* mosquito can then spread the virus to healthy humans when it injects saliva into those humans during the bite.

EPA Responses to Comments in Unit IX.D. — Comments Arguing that OX5034 is Efficacious. With regard to comments arguing that OX5034 will prove efficacious, the testing proposed to occur under the EUP is expected to be used in a subsequent registration application at which time EPA would determine how efficacious OX5034 is in suppressing *Ae. aegypti* mosquito populations in the United States where such data are required for control of public health pests. Determination of whether the product OX5034A is efficacious or not is one of the primary purposes of the EUP and is irrelevant to the decision to approve the EUP itself. Further, while EPA has reviewed information pertaining to the efficacy of OX513A, it is not relevant to the efficacy determination for OX5034 which will be evaluated as an isolated active ingredient.

X. Comments on Trial Parameters

Some commenters offered recommendations on appropriate design for the testing protocol that would be implemented under the EUP. These comments revolved around: (1) concerns that insufficient information had been provided to the public to allow meaningful public comment; (2) suggestions on specific aspects of trial parameters; and (3) suggestions that the testing should be performed in areas other than those described in the EUP application.

A. Comments Expressing the Opinion that Insufficient Information Had Been Provided to the Public

Some commenters (0023, 0130, 0327, 0342) argued that not enough information had been provided to the public to allow the public to comment on trial parameters.

W. Jordan and A. Jones (0327), noting that in a “Federal Register Notice signed on September 5, 2019, the Agency announced the receipt of an application for an Experimental Use Permit (EUP)

to evaluate the efficacy of releasing genetically engineered (GE) mosquitoes as a tool for suppression of wild *Aedes aegypti* mosquito populations", stated that:

> " . . . the public should be able to comment on the details of the actual studies proposed." (W. Jordan and A. Jones 0327 p. 1)

Explaining their position, W. Jordan and A. Jones (0327), added that:

> "The only document available for review on Regulations.gov is a letter from the applicant's attorney that describes a very general approach for the proposed Experimental Use Permit. To enable the public to provide meaningful input, the non-confidential portions of the actual EUP application should be available for comment. . . . In absence of information about the design of the efficacy research to be carried out under the EUP, it's difficult for the public to comment in a meaningful way." (W. Jordan and A. Jones 0327 p. 1)

Friends of the Earth (0342) specified that:

> " . . . there is insufficient data . . . , about the sites proposed for release in either Florida or Texas, and about Oxitec's proposed experimental program." (Friends of the Earth 0342 p. 1)

Anonymous (0023) and Anonymous (0130) stated that:

> "The proposal does not have sufficient information for the public to provide knowledgeable comments. For example, this experiment should have the methodology, planned statistical analysis, adaptive management, etc. for the public to review and comment. Simply stating a brief paragraph about the proposal is not enough information for individuals to analyze the approach of the proposed experiment." (Anonymous 0023 p. 1; Anonymous 0130 p. 1)

B. Comments Offering Suggestions on Specific Aspects of the Trial Parameters

Some commenters (0327, 0226, 0235, 0335, 0344) offered suggestions on how the trial should be conducted.

W. Jordan and A. Jones (0327) stated that because the release involves a significant public health pest:

" . . . , testing done under this proposed EUP should include a detailed, rigorous assessment of the efficacy of the proposed use of GE mosquitoes as a technique for suppression of wild populations." (W. Jordan and A. Jones 0327 p. 1)

To that end, W. Jordan and A. Jones (0327) made the following suggestions:

"If appropriate, EPA should direct the use of multiple methods of obtaining population counts to enhance confidence in the evaluation of efficacy." (W. Jordan and A. Jones 0327 p. 2)

"If approved, the EUP should be carried out in a manner that clearly isolates the effect of the release of the GE mosquitoes from other nearby mosquito-control efforts such as wide-area adulticide sprays and mosquito larvae control programs or control efforts by private landowners. There should be an assessment of whether the EUP results could have been confounded by other mosquito-control efforts in the same general area." (W. Jordan and A. Jones 0327 p. 2)

"Mosquito populations naturally fluctuate over time and space due to a variety of factors. To assess the potential impact of environmental factors (e.g., land cover, rainfall, temperature) on wild population levels, the EUP should collect data in a manner that enables comparison of areas into which GE mosquitoes were introduced and similar, geographically proximate areas where there was no such release. The comparison should be a quantitative." (W. Jordan and A. Jones 0327 p. 2)

"The adequacy of the time-frames for measuring population levels should be specified by the applicant and addressed in the EPA science review." (W. Jordan and A. Jones 0327 p. 2)

"There should be measurements of the ratio of GE mosquitoes to non-GE mosquitoes in the insects collected to determine population levels." (W. Jordan and A. Jones 0327 p. 2)

"The prevalence or occurrence of the GE trait over time should be measured to assess how long it takes for the release of GE mosquitoes to affect wild population levels and the durability of the effectiveness of the release of the GE mosquito." (W. Jordan and A. Jones 0327 p. 2)

"There should be an assessment of the spatial distribution of the trait and whether that changes over time. For example, does the geographic range of the trait change, if at all, beyond the area of the initial release? If yes, how far and how quickly does the trait change spread (or narrow)?" (W. Jordan and A. Jones 0327 p. 2)

> "The EUP should describe the statistical analyses that will be used to assess the data collected." (W. Jordan and A. Jones 0327 p. 2)

> "The EUP application should contain, and EPA should assess, all specific information – including the design of any efficacy assessments and the results obtained – used by the applicant to conclude that the GE mosquitoes were 'successfully deployed' in other areas (Cayman Islands, Brazil, Panama)." (W. Jordan and A. Jones 0327 p. 1-2)

The Center for Food Safety (0344) and GeneWatch UK (0335) called for the following to be supplied to the public:

> "The GPS coordinates and other relevant details of the proposed release sites and the scientific protocols for the proposed trial." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 15-16)

> "A proposal for comprehensive post-release monitoring of the proposed releases and their potential impacts on the environment." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 15-16)

The Center for Food Safety (0344) and GeneWatch UK (0335) also called for the following to be supplied to the public:

> "Information about which existing control methods will continue to be applied during the proposed releases." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 15-16)

R. Marquant III (0235) stated that in the request is based on an expectation:

> " . . . of up to 6600 acres at 20000 mosquitoes per acre or 132,000,000 mosquitoes per week with no statement on number of weeks the experiment will last nor how Oxitec will deal with unintended results or cross genetic mutations in the public summary. " (R. Marquant III 0235 p. 1)

Anonymous (0226) expressed concern that unanticipated incidents could give rise to unanticipated risks:

> ". . . , In caged experiments in Mexico using an earlier female-killing version (Oxitec's flightless female GM mosquitoes), the GM mosquito line was reportedly contaminated, so that half the GM females could fly and mate, rather than being unable to survive and reproduce." (Anonymous 0226 p.13) [Footnote omitted]

C. Comments Suggesting That Testing Be Performed in Areas Other Than Those Proposed in the EUP

EPA received seven comments questioning why certain areas had been selected as being appropriate sites or arguing that testing should be perform in areas other than those proposed in the EUP application. (0016, 0023, 0130, 0151, 0209, 0218, 0266). Most of these commenters argued that the sites chosen for the EUP testing are unsuitable for such testing primarily because of the number of humans residing in or visiting the test locations.

Anonymous (0023) and Anonymous (0130) stated that:

> ". . . , there is no information regarding why these test areas were selected. It seems odd that Monroe County Florida, a major tourist destination with over 112.8 million tourists each year from around the world (citation: Floridakeystreasures.com) was selected for a test location. This area is limited in space, which means that these test populations will be actively interacting with Florida Keys residents and tourists. There is not enough land to segregate them from the human population. By releasing an unknown experimental mosquito to this area, you are putting millions of people in direct risk and subsequently the entire country, since the majority of these people will travel home. Many tourists drive vehicles down to the Keys and have the potential to trap these mosquitos inside their vehicles, releasing it in an area outside of the test area. Has the Applicant shown sufficient evidence that the mosquitos will remain within the designated test area? It seems unlikely that they will with the amount of human influence in this area. The Applicant needs to select areas with little opportunity for human interaction or demonstrate scientifically that there is 0 risk to the human and natural environment." (Anonymous 0023 p. 1; Anonymous 0130 p. 1)

Anonymous (0209) stated that:

> "I totally object to the testing of this pesticide in the Fl. Keys. There are other places that are not inhabited (Everglades, forests, etc.) that are not populated by people where they can test their product. The Fl. Keys are fully inhabited. It makes no sense to test in a highly populated area." (Anonymous 0209 p. 1)

Anonymous (0016) stated that:

> "I ask that you diligently deny the permit, ask for much more time and scrutinize with objective fact checkers all their data, and additionally require they find a safe and unpopulated location, or better yet build a biodome to create the most basic of scientific base lines, that being in this case, a potential ground zero." (Anonymous 0016 p. 1)

D. Comments on the Potential for Mosquitoes to Move Away from the Test Area

Some commenters (0052, 0130, 0329, 0335, 0344) expressed concern that released OX5034 male mosquitoes might move away from the test area to surrounding areas.

Anonymous (0130) stated that:

> "Has the Applicant shown sufficient evidence that the mosquitos will remain within the designated test area? It seems unlikely that they will with the amount of human influence in this area." (Anonymous 0130 p. 1)

The Center for Food Safety (0344) and GeneWatch UK (0335) pointed out that if any of these mechanisms allow female *Ae. aegypti* larvae to mature to adults:

> "Eggs may survive for several months when dried out on the inner walls of containers and may be transported elsewhere. Any assessment therefore needs to consider the potential global transport of such eggs, and not be limited to considering the lifespan of adults and dispersal through adult flying." (Center for Food Safety 0344 p. 8; GeneWatch UK 0335 p. 7) [Footnote omitted]

Anonymous (0329) cautioned that "Once released, GM mosquitoes cannot be contained":

> "Aedes aegypti can travel up to 3 miles in certain conditions and even to other countries if they enter a vehicle, placing them well outside of any city or county they are released in." (Anonymous 0329 p. 2)

Anonymous (0329) also noted that:

> "Aedes aegypti are able to survive in cold weather.
> Aedes aegypti are able to breed in water with high levels of salinity.
> Aedes aegypti eggs can remain viable for up to 450 days." (Anonymous 0329 p. 2)

EPA Response to Unit X. A. — Comments Expressing the Opinion that Insufficient Information Has Been Provided to the Public. With regard to the comment that insufficient information was provided to enable the public to comment on the parameters of the field trial, 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. EPA previously

published and accepted public comment on a NOR for the OX513A mosquitoes in docket EPA-HQ-OPP-2017-0756.

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),
>
> (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.

With regard to the comment that EPA should require the company to submit certified data, EPA requires that data submitted to support either a registration or an EUP application comply with the requirements of Good Laboratory Practices Standards (GLP). Part 160 of Title 40 of the CFR describes good laboratory practices for conducting studies that support or are intended to support applications for research or marketing permits for pesticide products regulated by the EPA. This part is intended to ensure the quality and integrity of data submitted to the EPA pursuant to FIFRA sections 3, 4, 5, 18 and 24(c) and section 408 of the FFDCA.

EPA Response to Unit X.B. — Comments Offering Suggestions on Specific Aspects of the Trial Parameters. With regard to comments offering suggestions on what would be appropriate trial parameters, the document entitled "Review of Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 *Aedes aegypti* Mosquitoes Decision #549240; Submission #1047971" describes the EPA's evaluation of the trial parameters. That document can be found

in the docket for this action (EPA-HQ-OPP-2019-0274).[11] More detail about the parameters and experimental protocol can also be found in the Section G Experimental Program, as submitted by the registrant. The Section G Experimental Program can be found in the docket for this action.

With regard to comments expressing concern that unanticipated incidents might occur in the insectaries, EPA has reviewed the rearing protocol and found it to be adequate with appropriate quality control requirements, which will ensure that the *Ae. aegypti* males and eggs released are those that are described in the EUP application and evaluated by EPA in the risk assessment.

EPA Response to Unit X.C. — Comments Suggesting That Testing Be Performed in Areas Others Than Those Proposed in the EUP Application. With regard to comments suggesting that testing be performed in areas other than those proposed in the EUP application, efficacy testing on OX5034 cannot be conducted in an area away from humans. OX5034, the subject of the trial, is an *Ae. aegypti* mosquito. This species is highly anthropophilic, i.e., their preferred host is humans. Populations of this species are highest in areas where humans are present and lowest in areas where humans are less common. In the case of the Everglades or a biodome or other contained system, *Ae. aegypti* populations may not be adequate to evaluate the efficacy of the OX5034 mosquito. Thus, testing in such areas would not yield the necessary data/information.

EPA Response to Unit X.D. — Comments on the Potential for Mosquitoes to Move Away from the Test Area. With regard to the comment questioning whether OX5034 mosquitoes will remain in the test area, dispersal of OX5034 *Ae. aegypti* will be evaluated in trial A to determine how far the OX5034 strain of *Ae. aegypti* will disperse to provide information to inform product coverage, e.g., to determine efficacious placement of egg release boxes. For more information on trial A, see the assessment entitled "Review of Section G" that can be found in this docket. 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 m to 50 m of the release point, with a small percentage found 170 m but generally not more than 200 m from the release point[12]. This is in agreement with previous field releases of OX5034 male mosquitoes in Brazil that have recorded maximum dispersal distances of 198 meters, and average dispersal for the OX513A strain which is genetically similar to OX5034 is under 60 meters. Should mosquitoes be transported or otherwise dispersed beyond the test area, OX5034 is not expected to establish in areas outside of the test area for the same reason it is not expected to establish within the test area as discussed in EPA's response to Unit VI.A. This is because the OX5034 trait is self-limiting and thus is expected to be eliminated from the

[11] Hereafter referred to as "Review of Section G". This document can be found in the docket established for this action (EPA-HQ-OPP-2019-0274)

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

Ae. aegypti population regardless of whether that population is within or outside of the test area.

XI. Comments Calling for Additional Testing Before Releases are Permitted

Some commenters stated a belief that additional data/information should be developed, submitted and/or reviewed before EUP testing of OX5034 was permitted. (0005, 0028, 0041, 0046, 0047, 0052, 0053, 0054, 0056, 0059, 0071, 0076, 0078, 0079, 0088, 0092, 0096, 0107, 0111, 0114, 0119, 0128, 0140, 0141, 0143, 0151, 0168, 0173, 0178, 0187, 0198, 0214, 0221, 0232, 0233, 0245, 0259, 0271, 0272, 0273, 0277, 0290, 0302, 0308, 0314, 0315, 0317, 0318, 0320, 0329, 0331, 0334, 0335, 0342, 0344). Requests for such testing in some instances were specific as to which types of information/data the commenter thought should be developed. Others were not. This unit lists the specific comments and provides the title of the Unit of this Response to Comment document to which the comment appears to be most relevant.

A. Calls for Additional Testing on the Potential Consequences of OX5034 Genes Introgressing into the Local Wild *Aedes aegypti* Mosquito Population

Some commenters (0302, 0317, 0335, 0344) expressed the opinion that additional testing on the possibility of OX5034 genes introgressing into the local wild *Ae. aegypti* mosquito population, and the potential consequences of such introgression, should be conducted.

GeneWatch UK (0335) and Center for Food Safety (0344) stated that the studies needed to evaluate potential consequences of OX5034 genes introgressing into the local wild mosquito population would include:

> "Full independent testing of the non-native strain proposed for release, for disease transmission traits for all relevant diseases and insecticide resistance for all relevant insecticides, plus contained studies addressing concerns about the potential 'hybrid vigour' of any hybrid strains." (GeneWatch UK 0335 p. 15; Center for Food Safety 0344 p. 18)

The Center for Food Safety (0344) and GeneWatch UK (0335) argued that necessary studies include:

> "A full, published investigation into the reported survival of hybrid GE mosquitoes in Brazil, including a specific investigation of the recent open release trials of OX5034 GE mosquitoes. This study should include detailed analysis of any hybrid mosquitoes for disease transmission properties." (GeneWatch UK 0335 p. 16; Center for Food Safety 0344 p.18)

Anonymous (0302) stating that Oxitec has not performed any epidemiological trials commented that:

> "Results from epidemiological trials remain the primary missing information for assessment of the public health value of this product, . . ." (Anonymous 0302 p. 1)

B. Calls for Additional Testing on Whether Adult OX5034 Female Mosquitoes or Their Offspring Females Expressing OX5034's Engineered Genes Might Occur in the Test Areas

Several commenters (0335, 0342, 0344) requested that additional testing be performed and independently replicated on whether biting female OX5034 mosquitoes could be present in the test area.

The Center for Food Safety (0344) and GeneWatch UK (0335) stating that there "are no published peer-reviewed paper for Oxitec's GE *Aedes aegypti* OX5034 mosquitoes," indicated that necessary tests include:

> "• Independent verification that the new OX5034 strain provides Oxitec's claimed "*genetic separation to 100% males*": plus estimates of the numbers of GE biting female mosquitoes that may be released during the proposed experiments, or that may survive from subsequent generations, taking into account the potential to encounter tetracycline in the environment.
>
> • Studies of the potential of the GE mosquitoes to evolve resistance to the killing mechanism during mass breeding or following release, plus studies of the potential for wild females to evolve behavioural resistance.
>
> •Identification of potential sites where GE mosquitoes could encounter industrially farmed meat (e.g. discarded takeaways, pet food) and testing of tetracycline levels at these sites.
>
> •A full, published investigation into the reported survival of hybrid GE mosquitoes in Brazil, including a specific investigation of the recent open release trials of OX5034 GE mosquitoes.
>
> •A full, published investigation into the unexpected survival of female mosquitoes in Oxitec's experiments in Mexico." (Center for Food Safety 0344 p. 18)

The Center for Food Safety (0344) and GeneWatch UK (0335) added that necessary tests included:

> "Identification of relevant septic tanks and cess pits where mosquitoes may breed and testing of tetracycline levels in them." (GeneWatch UK 0335 p. 15-16)

Friends of the Earth (0342) specified that:

> " . . . there is insufficient data about the sterility of the OX5034 mosquitoes, about the sites proposed for release in either Florida or Texas, and about Oxitec's proposed experimental program." (Friends of the Earth 0342 p. 1)

Friends of the Earth (0342) urged EPA while evaluating OX5034 to pay particular attention to:

> "•The risks of releasing biting females
>
> • Potential locations where significant levels of tetracycline may be present.
>
> • The risks associated with mosquitoes surviving into adulthood if tetracycline is present in the surrounding environment." (Friends of the Earth 0342 p. 7)

Gene Watch UK (0335) and Center for Food Safety (0344) noted that:

> " . . , it is unclear whether or not tTAV-OX5034 is the identical to the protein in the OX513A strain, and no studies specific to the OX5034 strain have been provided." (GeneWatch 0335 p. 14; Center for Food Safety 0344 p. 16)

GeneWatch UK (0335) and Center for Food Safety (0344), argued that additional testing is necessary:

> "Considerably more data, based on specific feeding trials in relevant species, is therefore needed to establish that consumption of OX5034 GE mosquito adults or larvae is not harmful to humans, farm animals, pets or wildlife." (GeneWatch 0335 p. 14; Center for Food Safety 0344 p. 17)

GeneWatch UK (0335) and Center for Food Safety (0344) referred EPA to the standards developed by the European Union (EU) for GE insects indicating that these state:

> " . . . (page 8): "…*applicants should also assess the likelihood of oral exposure of humans to GM animals or their products which are not intended for food or feed uses. If such exposure is likely and ingestion or intake will occur at levels which could potentially place humans at risk, then applicants should apply the assessment procedures described in the EFSA Guidance Document on the risk assessment of food and feed from GM animals and*

on animal health and welfare aspects". To meet the requirements of the cited Guidance on risk assessment of food and feed, it is likely that repeated dose toxicity studies using laboratory animals would be required." (GeneWatch UK 0335 p. 14 Center for Food Safety 0344 p.17) [Emphasis in the original] [Footnote omitted]

The Center for Food Safety (0344) and GeneWatch UK (0335) called for:

> "Publication of laboratory studies, including studies of proteins in mosquito saliva, . . ." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 16)

Friends of the Earth (0342) requested "independent replication of Oxitec's laboratory results":

> " . . . including studies of proteins in mosquito saliva . . . ." (Friends of the Earth 0342 p. 7)

C. Calls for Additional Testing on Environmental Considerations: Effects on the Food Supply and Population Dynamics

Comments received revolved around concerns that insufficient testing of the effects OX5034 mosquitoes might have on other organisms does not give the Agency the information needed to adequately assess potential risks. (0329, 0335, 0342, 0344). Commenters stated specifically that additional testing should be done to examine potential effects on organisms that might consume mosquitoes, and on dynamic changes in organism populations.

1. Potential Effects on Organisms That Might Consume Mosquitoes

Some commenters (0329, 0335, 0342, 0344) voiced concern that manipulating the *Ae. aegypti* population might affect organisms that consume mosquitoes as food.

Anonymous (0329) stated that:

> "GM mosquitoes have not been adequately tested for toxicity in Florida and Texas native animals, including endangered species, that could consume them." (Anonymous 0329 p. 2)

The Center for Food Safety (0344) and GeneWatch UK (0335), stating that there "are no published peer-reviewed paper for Oxitec's GE *Aedes aegypti* OX5034 mosquitoes", indicated that necessary tests included:

> "Laboratory safety tests, including feeding trials for relevant wild species and laboratory rats to better establish the claim of no harmful effects of ingestion and/or biting." (GeneWatch 0344 p. 15-16)

Friends of the Earth (0342) requested "independent replication of Oxitec's laboratory results" on:

> " . . . feeding trials . . . ." (Friends of the Earth 0342 p. 7)

While recognizing that Oxitec has published one feeding study, in which OX513A GE *Ae. aegypti* mosquito larvae were fed to two different species of a type of mosquito that eats other mosquitoes (known as *Toxorhynchites*), and that Oxitec published a feeding study on the impact of GE olive flies on one parasitoid (a wasp) and two predators (a spider and a beetle), that reported no adverse effects, GeneWatch UK (0335) and the Center for Food Safety (0344) expressed concern that in the context of the OX5034 application:

> "As far as we are aware, no feeding trials have been published which study potential impacts on birds, mammals, reptiles or amphibians, such as lizards or frogs. Further, no independent studies have been published." (GeneWatch 0335 p. 14; Center for Food Safety 0344 p. 16)

Friends of the Earth (0342) stated that:

> "It is unclear what the impacts of the GE mosquitoes on wild animals, including endangered or threatened species, and farm animals are. More feeding trials are needed to assess the risk of ingestion to wild species that eat mosquitoes. We also need adequate assessment of the potential impacts of increased mosquito populations when the trial GE mosquitoes are initially released on ecosystems, particularly in the surrounding areas where the mosquitoes would be released and could spread. GeneWatch UK's previous public comments on the OX513A are still relevant and note that increases in non-target mosquito species as a result of the proposed releases could pose risks to human and animal health, as could increases in the target species in areas neighboring the releases." (Friends of the Earth 0342 p. 3) [Footnote omitted]

GeneWatch UK (0335) and Center for Food Safety (0344), noting that for "biopesticides, the EPA typically requires Tier I testing done on the following non-target organisms: birds (oral and inhalation), mammals, freshwater fish and invertebrates, estuarine/marine fish and invertebrates, plants, insects, and honeybees. Tier II, III, and IV testing is triggered only when unacceptable effects are seen at the Tier I testing level," stated that:

> "Considerably more data, based on specific feeding trials in relevant species, is therefore needed to establish that consumption of OX5034 GE mosquito adults or larvae is not harmful to humans, farm animals, pets or wildlife. . . , wildlife consuming the GE mosquitoes may also include threatened or endangered species, and this risk also needs to be assessed." (GeneWatch 0335 p. 14; Center for Food Safety 0344 p. 17)

To reinforce the argument that more testing is needed, GeneWatch UK (0335) referred EPA to the guidance developed by the European Union (EU) for GE insects indicating that:

> "EU Guidance on risk assessment of GE insects (known as GM insects in Europe) published by the European Food Safety Authority (EFSA) requires applicants to assess the effects of toxins or allergens associated with the GE insect in animals such as birds, mammals, reptiles and amphibians." (GeneWatch UK 0335 p. 14) [Footnote omitted]

Center for Food Safety (0344) clarified that:

> "European Union (EU) standards are relevant here because Oxitec is required by EU law to provide a risk assessment which meets EU standards to the importer, before exporting its GE mosquitoes." (Center for Food Safety 0344 p. 17) [Footnote omitted]

Center for Food Safety summarized their arguments by stating that:

> "In summary, considerable further evidence is needed to assess whether the use of the pesticide under the proposed permit (including its method of delivery) may cause unreasonable adverse effects on the environment. . . . It is notable that information supplied to the various review processes for OX513A GE mosquitoes and other insects (to the FDA, APHIS and the EPA, as well as overseas agencies) is almost entirely lacking in the current process for the proposed release of the new generation of OX5034 GE mosquitoes." (Center for Food Safety 0344 p. 17)

The Center for Food Safety (0344) and GeneWatch UK (0335) called for:

> "Publication of laboratory studies, including . . . feeding trials with mosquito predators . . . ." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 16)

2. <u>Dynamic Changes in Organism Populations</u>

Some commenters (0335, 0342, 0344) argued that the Agency must evaluate the potential for dynamic changes in local ecosystems as a result of the proposed releases.

The Center for Food Safety (0344) and GeneWatch UK (0335) called for:

> "Further consideration of the dynamic changes in local ecosystems as a result of the proposed releases, including the impacts of a large (potentially several orders of magnitude) increase in the number of adult mosquitoes in the target area during the releases." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 16)

The Center for Food Safety (0344) and GeneWatch UK (0335) called for:

> "Full review of risk of increasing other mosquito vectors, including: laboratory and caged trials on the impacts of interspecies competition; thorough baseline studies of mosquito populations; studies on the disease transmission properties of other vectors for all relevant diseases; and consideration of the possibility that viruses will evolve in response to ecosystem changes." (Center for Food Safety 0344 p. 18; GeneWatch UK 03353 p. 15-16)

GeneWatch UK (0335) stated that:

> "According to the summary of the application, the proposed experiments are to evaluate the efficacy of OX5034 mosquitoes as a tool for suppression of wild *Aedes aegypti* mosquito populations. . . . However, many more such studies (in contained use, and by monitoring and modelling the behaviour of wild mosquito populations and their ecosystems) would be required before an adequate risk assessment could be undertaken." (GeneWatch UK 0335 p. 12)

The Center for Food Safety (0344) and GeneWatch UK (0335) also called for:

> "Full review of the risk of interfering with other mosquito control systems such as the *Wolbaccia* [sic] infected *A. albopictus* trials." (Center for Food Safety 0344 p. 18; GeneWatch UK 03353 p. 15-16)

Finally, the Center for Food Safety (0344) and GeneWatch UK (0335) called for:

> "Further consideration of the dynamic changes in local ecosystems as a result of the proposed releases, including the impacts of a large (potentially several orders of magnitude) increase in the number of adult mosquitoes in the target area during the releases." (Center for Food Safety 0344 p. 18; GeneWatch UK 03353 p. 15-16)

Friends of the Earth (0342) called for assessments that looked at:

> "The ecological risks of released GE mosquitoes including the impacts on food chains or opening new ecological niches for more dangerous insects to replace the *Aedes aegypti"* (Friends of the Earth 0342 p. 7)

Friends of the Earth (0342) also called for assessments that looked at:

> "Potential environmental and health impacts of releasing millions of mosquitoes on a regular basis." (Friends of the Earth 0342 p. 7)

Finally, Friends of the Earth (0342) also called for assessments that looked at:

> "Alternatives to using GE mosquitoes as a way to limit the spread of dengue fever such as bed nets, community-based prevention programs, and other biological tools that do not depend on expensive and risky genetic engineering technologies." (Friends of the Earth 0342 p. 7)

D. Calls for Additional Testing on Potential for Environmental Release of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations

Some comments (0300, 0331, 0334, 0335, 0344) called for additional testing to address the potential for OX5034 mosquito releases to increase antibiotic resistance in microbial populations.

Center for Food Safety (0344) and GeneWatch UK (0335) stated that considerably more information is needed to confirm or rule out the presence of antibiotic resistant bacteria in the GE mosquitoes intended for release. For example, Center for Food Safety (0344) stated that:

> "More information is needed to be able to confirm or rule out the presence of such antibiotic resistant bacteria in the GE mosquitoes intended for release. Antibiotic resistant bacteria could pose a major risk to health if spread into the environment." (Center for Food Safety 0344 p. 10; most of this comment repeated by GeneWatch UK 0335 at p.8)

J.W. Norris (0334) stated that:

> "Our petition continues to request culture testing to assess this human health concern." (J.W. Norris 0334)

The Florida Keys Environmental Coalition (0331) stated that:

- "Mosquitoes, eggs and larva need to be submitted for independent antibiotic resistant bacteria evaluation by more than one objective agency, or qualified entity." (Florida Keys Environmental Coalition 0331 p. 3)

The Center for Food Safety (0344) and GeneWatch UK (0335) stating that there "are no published peer-reviewed paper for Oxitec's GE *Aedes aegypti* OX5034 mosquitoes," indicated that necessary tests included:

> "Laboratory studies of the potential for antibiotic resistant bacteria to be spread into the environment via adult mosquito releases or disposal of larval rearing water or other wastes from the mosquito production facility." (GeneWatch UK 0335 p. 15-16; Center for Food Safety 0344 p. 18)

The Center for Food Safety (0344) and GeneWatch UK (0335) called for:

> "Publication of laboratory studies, including . . . larval survival rates in the presence and absence of tetracycline contamination." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 16)

Friends of the Earth (0342) requested "independent replication of Oxitec's laboratory results" on:

> " . . . larval survival rates in the presence of tetracycline contamination. . . ." (Friends of the Earth 0342 p. 7)

J.W. Norris (0334) said that physicians in the Florida Keys for OX513A and now for OX5034 want to:

> " . . . characterize resistance among the isolated microbes from OX513A to-be-released mosquitoes; we also wanted to swab and culture trays actively being used to manufacture the OX513A. However, a very inexpensive option would have been to use sterile forceps to grasp individual OX513A mosquitoes and smear them on culture media." (J.W. Norris 0334 p. 2 of the Attachment)

E. Calls for Additional Testing for Efficacy

Some commenters (0005, 0317, 0335, 0342, 0344) thought the Agency should require additional efficacy testing.

The Center for Food Safety (0344) stated that:

> "To date, Oxitec has not established the efficacy of its technique for reducing *Aedes aegypti* populations, or the impact on relevant diseases (which may continue to be transmitted even by relatively small numbers of mosquitoes, including other species). Existing data from experiments elsewhere suggests the efficacy of this approach is poor . . . and there is no efficacy data for the United States. Further efficacy data would therefore certainly be needed before Oxitec could register its GE mosquitoes as a pesticide under 7 U.S.C. 136a. Based on the paucity of efficacy and other data . . . further studies are first essential to establish that the proposed experimental use will not cause unreasonable adverse effects on the environment." (Center for Food Safety 0344 p. 14)

GeneWatch UK (0335) made a similar comment, noting that:

> " . . . GeneWatch UK opposes the granting of the experimental use permit, as further studies are first essential to establish that the proposed experimental use will not cause unreasonable adverse effects on the environment. . . ." (GeneWatch UK 0335 p.12)

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that studies necessary to fully evaluate OX5034 include:

> "Published confirmation and independent verification of Oxitec's claim that its trial of OX5034 in Brazil has been successful . . . ." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 17)

Anonymous (0005) and D. Rubin (0317) stated that:

> "The applicant failed to submit any certified data arising out from the use of this product in Brazil, Panama and elsewhere; indicating the effectiveness and long term safety to humans, and other species. Therefore, EPA should request such certified data in order to seriously consider the application." (Anonymous 0005 p. 1; D. Rubin 0317 p. 1)

Friends of the Earth (0342) stated that:

> " . . . , although Oxitec claims that the GE mosquito could reduce rates of dengue fever by reducing *Aedes aegypti* mosquito populations, it is uncertain that, even if *Aedes aegypti* mosquito populations were reduced, there would be a reduction in rates of disease as other mosquitoes also carry dengue, zika, and related viruses. Oxitec has not provided data or evidence to assess whether these population *Aedes aegypti* reductions would lead to disease eradication or reduction." (Friends of the Earth 0342 p. 2)

Friends of the Earth (0342) further stated that:

> "Even in its trials in Grand Cayman, the company did not demonstrate that reducing overall populations of mosquitoes will reduce or eradicate disease, as dengue is not endemic in the Cayman Islands. As suggested by Center for Food Safety in its public comments, Oxitec should provide a specific mechanism through which its proposed releases might reduce the risk of diseases spread through mosquitoes in the Florida Keys and Harris County, Texas. Without this information, Oxitec's proposed "pesticide" experiment will not address disease reduction." (Friends of the Earth 0342 p. 4) [Footnote omitted]

EPA Response to Unit XI. – Comments Calling for Additional Testing. With regard to comments calling for additional testing, most of the concerns expressed in these comments revolve around a lack of publicly available information on OX5034, e.g., published studies on various aspects of OX5034 behavior. The EPA carefully examined the data and information submitted to the Agency in support of the EUP request. The details of that assessment can be found in the Human Health and Environmental Risk Assessment, and the Review of Section G. These documents can be found in the docket established for this action (EPA-HQ-OPP-2019-0274).

With regard to the request for independent replication of Oxitec's laboratory results, EPA has not historically required any applicant to provide independent replication of their laboratory results. Rather, the Agency requires that data submitted to support either a registration or an EUP application must comply with the requirements of Good Laboratory Practices Standards (GLP). Part 160 of Title 40 of the CFR describes good laboratory practices for conducting studies that support or are intended to support applications for research or marketing permits for pesticide products regulated by the EPA. This part is intended to ensure the quality and integrity of data submitted to the EPA pursuant to FIFRA sections 3, 4, 5, 18 and 24(c) and section 408 of the FFDCA. EPA further notes to the regulations found at 40 CFR 158 that the Agency relies on to describe the kinds of data and information EPA requires in order to make judgements under FIFRA sections 3, 4 and 5 about the risks and benefits of pesticide products, and if pesticide residues may occur in food or feed due to use of the pesticide to determine the safety of pesticide chemical residues under FFDCA section 408.

With regard to comments pointing EPA to the guidance developed by the European Union (EU) for GE insects published by the European Food Safety Authority (EFSA) that requires applicants to assess the effects of toxins or allergens associated with the GE insect in animals such as birds, mammals, reptiles and amphibians, EPA declines commenters' invitation to assess whether Oxitec has met whatever obligations it may have to the European Union (EU) under European Food Safety Authority (EFSA) guidance. Whatever such obligations may be, they would be owed by Oxitec to the EU; they are neither owed to nor enforceable by EPA.

With regard to the comment questioning the impact of reducing *Ae. aegypti* mosquito populations on relevant diseases, because the OX5034 mosquitoes are intended for suppression of *Ae. aegypti* mosquito populations and are not intended to directly influence disease transmission, epidemiological studies assessing effects on disease transmission are not required to support this product for registration under FIFRA section 3.

With regard to the comment that additional efficacy data would be needed before Oxitec could register its GE mosquitoes as a pesticide under 7 U.S.C. 136a, EPA respectfully notes that the purpose of this Experimental Use Permit is to generate efficacy data within the United States necessary to support a future section 3 registration action. Efficacy data do not need to be submitted to support an experimental use permit. However, the experimental protocol in the EUP must be designed using appropriate scientific methods to collect efficacy data on the pesticide(s) to be tested.

With regard to the comment that the EUP application to permit the testing of OX5034 should contain, and EPA should assess, all the data and information used by the applicant to conclude that the GE mosquitoes were indeed successfully deployed in areas such as the Cayman Islands, Brazil, and Panama, as previously noted, efficacy data are not required to be submitted for an EUP application. EPA evaluated information submitted to support the experimental design for the EUP application and also availed itself of information in the published literature. Although methods and results from previous studies on GE mosquitoes other than OX5034 can be helpful for developing and evaluating test protocols for an EUP, efficacy of other GE mosquitoes is not relevant to EPA's decision on issuance of the Experimental Use Permit under FIFRA section 5 for OX5034. Efficacy of OX5034 will be determined by data submitted in support of a subsequent FIFRA section 3 registration application.

With regard to the purpose of an EUP, Section 5(a) of FIFRA, 7 U.S.C. § 136c(a), states, in part: "The Administrator may issue an experimental use permit only if the Administrator determines that the applicant needs such permit in order to accumulate information necessary to register a pesticide under section 3 of this Act." The "Data Requirements for Pesticides" at 40 CFR Part 158 state, in part: "Product performance data must be developed for all biochemical pesticides. However, the Agency typically does not require applicants to submit such efficacy data unless the pesticide product bears a claim to control … invertebrates (including but not limited to: mosquitoes and ticks) that may directly or indirectly transmit diseases to humans." 40 CFR 158.2070. As EPA stated in the Notice of Receipt (NOR) of the EUP application published in the Federal Register: "The proposed experiments are to evaluate the efficacy of OX5034 mosquitoes as a tool for suppression of wild Aedes aegypti mosquito populations." 84 Fed. Reg. 47,947 (Sept. 11, 2019). Oxitec needs the present EUP in order to accumulate data regarding efficacy / product performance that is necessary to an application for registration under Section 3 of FIFRA. This is an appropriate purpose of an EUP.

Further, the EUP regulations at 40 CFR Part 172 state, in part: "Refusal. At any time that the Administrator determines that an experimental use permit is not justified, or that the issuance of such a permit would cause unreasonable adverse effects on the environment, or that for any other reason provided for under the law a permit shall not be issued, he shall notify the applicant in writing." 40 CFR 172.10(a). As fully explained in the Human Health and Environmental Risk Assessment, which can be found in this docket (EPA-HQ-OPP-2019-0274), EPA has found that the permit will not cause unreasonable adverse effects on the environment, including human health. See Unit III "Human Health & Environmental Risk Conclusions" for additional information.

XII. Comments Recommending Actions EPA Should Take

Comments were received recommending actions that the EPA should take with regard to the request for an EUP to permit testing of OX5034. Most of these comments revolve around: (1) requests that the Agency be transparent in its decision making; (2) the public's desire for opportunity to be part of the decision making process, including ensuring that the public has opportunity to review the data supporting the request; (3) the public's desire that the Agency ensures that scientific expertise external to the EPA has opportunity to review the data supporting the request; and (4) requests that the EPA put in place a mitigation strategy to use in the event OX5034 does not behave as anticipated.

A. Comments Arguing That EPA Should Extend the Comment Period

Five commenters (0038, 0243, 0290, 0326, 0344) requested an extension of the comment period allotted for the Notice of Receipt for an application requesting an EUP to evaluate the efficacy of releasing male OX5034 as a tool to suppress wild *Ae. aegypti* mosquito populations.

The Center for Food Safety (0344) stated that:

> "EPA should extend the comment period until the EPA and Oxitec can provide the public a more adequate set of data to review. At the very least, the public should be able to review all data generated from any caged trials of the new Oxitec OX5034 strain of the GE mosquito in any country. Oxitec should provide complete genomic sequence of the OX5034 strain, including both the intended genetic engineering and any off-target effects of the engineering. Oxitec provides no evidence that the female-killing mechanism engineered into the OX5034 strain is 100% effective. It is essential that such evidence is published and made available for independent scrutiny and consultation in order to assess the risk of release of female GE mosquitoes in the proposed experiments. Oxitec should provide all data on the survivability of the OX5034 strain, including how many females might have escaped from the caged confinement. If Oxitec has not studied which other *Ades* [sic] species and which other *Ades [sic] aegypti* strains the OX5034 strain can hybridize with after release, such experiments need to be

required given recent reports of its earlier strain hybridizing with wild type *A. aegypti*." (Center for Food Safety 0344 p. 3) [Footnote omitted]

Commenter B. Wray (0038) stated that:

> "The brevity of the comment period adds risk to the review process, and we ask that a reasonable extension of 60 days be granted to permit proper investigation and response to be accumulated from a broad array of sources. Without great objective input, novel advanced technology cannot be properly vetted prior to use." (B. Wray 0038 p.1)

Referring to the Evans et al paper, commenter B. Wray (0038) furthered the argument stating that:

> "After repeated visits to Regulations.gov the Docket still only has the EPA instructions and a general letter that suggests the available performance data is limited to a marketing document making claims with no measured quantified documented results to support Oxitec's performance claims. There is no long terms analysis on the actual reproductive legacy, genetic heredity, introgression and hybridization of other wild type species. In the wake of the Yale study, no releases of any Oxitec genetically modified species should be permitted without complete understanding of all unintended off-site mutations." (B. Wray 0038 p. 2)

Commenter B. Wray (0038) specifically noted that:

> "Zero analysis exists with regard to antibiotic resistant bacterial promotion." (B. Wray 0038 p. 1)

GMO Free USA (0326) requested an extension of the public comment period for an additional 90 days arguing that 30 days is simply not enough to evaluate the complexity and impacts of this proposal because:

> "1. New research has been published this week on the efficacy of a release of Oxitec's genetically engineered mosquitoes on mosquito populations in Brazil. The study, published by Yale University scientists in the journal Nature, documented unexpected and unintended consequences from the release.
> https://www.nature.com/articles/s41598-019-49660-6

> "2. The granting of this experimental permit has the potential, over time, to impact ecosystems across the country and those of neighboring countries. . . .

> "4. Extending the comment period is warranted due to the numerous legal, scientific and economic considerations contained within this proposal including potential allergenicity or other unintended effects on public health." (GMO Free USA 0326 p. 1)

B. Comments Arguing That EPA Should Offer an Additional Opportunity for Public Comment

Some commenters (0124, 0327) argued that EPA should offer an additional opportunity for public comment.

W. Jordan and A. Jones (0327) stated that:

> "Given the significance of the proposed EUP, we recommend that EPA open an additional round of comment that allows the public to see the proposal detailing the methods for evaluating efficacy under the EUP, together with EPA's scientific review of those methods." (W. Jordan and A. Jones 0327 p. 2-3)

Anonymous (0124), noting that several safety related points had not been addressed suggested that:

> "Oxitec be required to address these safety issues fully and then the public be given a further opportunity to comment." (Anonymous 0124 p. 1)

C. EPA Should Ensure Transparency

Some commenters (0035, 0295, 0342, 0344) raised issues that revolve around transparency of the Agency in its actions. Most of these commenters requested that the Agency reveal its analyses of the OX5034 proposal.

Friends of the Earth (0342) stated that:

> "Because this is a new genetically engineered insect to be reviewed as a pesticide, the EPA must reveal its analysis of the environmental, health and social impacts of Oxitec's GE mosquito release proposal;" (Friends of the Earth 0342 p. 8)

The Center for Food Safety (0344) stated that:

> "No public information has been provided in the Docket or elsewhere relating to the survival rates of GE females to adulthood, in the presence or absence of sources of tetracycline: this makes it impossible to assess Oxitec's claim that no biting GE females will be released or survive to adulthood." (Center for Food Safety 0344 p. 2)

The Center for Food Safety (0344) stated that:

> "The documents provided to EPA for the docket include no details of Oxitec's proposed experimental program, and no environmental assessment (EA) or environmental impact statement (EIS) has been provided. The Center for Food Safety and a coalition of groups gave the FDA notice that the coalition planned to sue under the Endangered Species Act given the arbitrary and capricious environmental review performed by the FDA." (Center for Food Safety 0344 p. 3) [Footnote omitted]

Commenter J. Barton (0295) stated that:

> "Since this is the first genetically engineered insect to be reviewed as a pesticide, the EPA must reveal its analysis of the environmental, health and social impacts of Oxitec's GMO mosquito release proposal;" (J. Barton 0295 p. 1)

Commenter M. Jones (0035) stated that while from "what I have learned about this issue, the procedure to find a way to attempt to downregulate the disease vector *Aedes aegypti* is a logically sound one overall":

> "I oppose implementation in the US; we need to be certain that the technology is reliable in field applications. I am very concerned that the timing also of this request is of such a short fuse that the issue will become largely unnoticed and the technology allowed to slip through and be implemented without sufficient public and scientific comment. . . . the research group must establish a stronger 'kill effect' and find ways to prevent the development of unforseen [sic] consequences." (M. Jones 0035 p. 1)

D. Comments Arguing That EPA Should Seek Advice From Independent Committees of Experts

Several commenters (0125, 0176, 0295, 0316, 0320, 0335, 0342, 0344) argued that EPA should seek advice on OX5034 from independent experts.

Friends of the Earth (0342) stated that EPA should:

> "Have a committee of independent ecologists and entomologists, public health experts (including dengue fever and zika virus specialists), and other key experts and public stakeholders review the proposal from Oxitec;" (Friends of the Earth 0342 p. 8)

Friends of the Earth (0342) also argued that a full EIS prepared for OX5034 should be:

> " . . . reviewed by a committee of independent ecologists and entomologists, public health experts, and other key experts and public stakeholders."(Friends of the Earth 0342 p. 2)

Friends of the Earth (0342) added that experts external to the Agency should also be present at meetings with the public for:

> " . . . review of the companys [sic] proposal . . . ;" (Friends of the Earth 0342 p, 8)

Commenter J. Barton (0295) stated that:

> "EPA must form a committee of independent ecologists and entomologists, public health experts (including dengue fever and zika virus specialists), and other key experts and public stakeholders to review the proposal from Oxitec;" (J. Barton 0295 p. 1)

K. Gould (0320) demanded that:

> ". . . the EPA receive more expert opinion to make sure that this procedure is valid. If it isn't, then the EPA must abandon this proposal." (K. Gould 0320 p. 1)

E. Comments Arguing that the Agency Should Seek Public Input

Several commenters (0045, 0107, 0176, 0236, 0295, 0316, 0322, 0342) requested that the Agency seek public input into the decision-making process. Some commenters requested that EPA convene meetings with the public, others proposed that a referendum be held.

Commenter Anonymous (0236) offered the opinion that:

> "As an American citizen, I feel that it should not be up to a small group of people to decide what to release into my environment. I do not consent to this experiment that could potentially impact my health and the health of others." (Anonymous 0236 p. 1)

1. Convene Meetings with the Public

Some commenters (0295, 0342) argued that EPA should convene meetings with the public to obtain input on the OX5034 proposal.

Friends of the Earth (0342) stated that EPA should:

> "Convene public meetings in the counties where site releases are planned, advertised in the Federal Register, for the review of the company's proposal with the above committee present;" (Friends of the Earth 0342 p. 8)

Friends of the Earth (0342) also stated that:

> "The EPA should also convene public meetings in sites of release as well as the areas surrounding the release site in Florida and Texas. These meetings should be advertised in the Federal Register, and Oxitec's data and EIS should be available for review." (Friends of the Earth 0342 p. 2)

Commenter J. Barton (0295) stated that:

> "The EPA must convene public meetings in Monroe County, FL (the Florida Keys) and Harris County, Texas, advertised in the Federal Register, for public comment and review of the company's proposal with the above committee present;" (J. Barton 0295 p. 1)

2. <u>Hold a Referendum</u>

Some commenters (0045, 0322) requested that a referendum on the question of whether testing might occur in designated areas should be held.

Anonymous (0045) stated that:

> "I reside in Key Haven, Florida. I am asking for another referendum on version 2 GM Mosquito. You can not[sic] release without our consent for a newer version with the same problems. We need more time to inform our neighbors to allow them to comment and to vote on being test subjets [sic]. I vote NO once again, and ask for more time to make sure our neighbors make informed decisions." (Anonymous 0045 p. 1)

Anonymous (0322) stated that:

> "Monroe County had a referendum question regarding an Oxitec trial in the Florida Keys in the most recent general election. The data is broken down by precinct, of course. The results are in favor of mosquito trials. I think a trial should take place in those areas that have expressed overwhelming approval of such a test." (Anonymous 0322 p. 1)

F. Comments Challenging OX5034 Mosquito Regulation Under FIFRA

Some commenters (0306, 0333) challenged EPA's authority to regulate OX5034 under the Agency's FIFRA authorities.

Commenter L. Sanders (0333) stated that:

> "Let it be known that the FIFRA does not include regulation or definition of a Genetically Modified Insect and can therefore not be used as justification for testing Mosquitoes as a pesticide. The definition of pesticide in the FIFRA is, (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. The substance to which Oxitec is referring to is 0.000056g active ingredient of tTAV-OX5034. This is a gene that is expressed when tetracycline is not present and the protein it creates helps make more tTA that kill the larvae. It however is not a pesticide and cannot be regulated by FIFRA. Also, the mosquito itself is being used as a pesticide and by definition cannot be called a pesticide. A mosquito is an insect. It is alive and this insect release should be looked at as though a foreign animal or plant were introduced into the ecosystem." (L. Sanders 0333 p. 1)

Commenter M. Hull (0306) argued that:

> "The unprecendented [sic] technology / patented life form, OX5034, requires regulatory jurisdiction from a bioethics regulatory body. In truth, the USDA admitted no jurisdiction, because this is not an agricultural product. The USFDA also admitted no jurisdiction, because this product is not a "veterinary drug." To be fair, this unique artificially modified subspecies of an invasive species, the *Aedes aegypti*, is not, as described, a "pesticide." There is no MSDS nor OSHA directive regarding this genetically modified insect as "pesticide. . . . For the Environmental Protection Agency to regulate this uniquely damaged animal is bizarre and unsuited to the purpose of this federal regulatory agency. Public health is at stake. Where are the CDC and NIH in this dialogue? Where is the World Health Organization in this debate? What about the tetracycline resistance and concerns of a growing number of physicians researching this technology with a level of scrutiny for public health . . . ?" (M. Hull 0306 p. 1)

G. Comments Arguing that EPA Needs to Develop New Regulations for GE Insects

Some commenters (0295, 0316, 0335, 0342, 0344) argued that EPA needs to develop new regulations specific for GE Insects. Some commenters were concerned that not only the active ingredient, but also the mode of delivery, the mosquito, needs to be part of EPA's regulatory approach to products such as OX5034. Some commenters argued that only after such regulations are in place should EPA consider applications from potential registrants.

Friends of the Earth (0342) and J. Barton (0295) stated that EPA should:

> "Develop new regulations for genetically engineered insects designed to be biopesticides -- only after these regulations are in place should EPA consider an application for GE insects." (Friends of the Earth 0342 p.2 and p. 8; J. Barton 0295 p. 2)

Friends of the Earth (0342) stated that the "EPA should issue new regulations that cover GE mosquitoes before it allows any experimental use of GE mosquitoes", given that:

> "No federal agency has formal regulations specific to GE insects and animals, or law that addresses the risks and all of the types of GE insects. The current U.S. regulatory system is outdated and lacks clear oversight of the use of biotechnology, particularly when it is used for proposals to eliminate insect vectors of animal and human diseases." (Friends of the Earth 0342 p. 5)

The Center for Food Safety (0344) and GeneWatch UK (0335) also called for the following to be supplied to the public:

> "Published criteria for assessing the impact of existing control measures and the proposed releases on the target pest and the risks of all the relevant diseases." (Center for Food Safety 0344 p. 18; GeneWatch UK 0335 p. 15-16)

Friends of the Earth (0342) further stated that:

> "Regulatory action under the Insecticide, Fungicide and Rodenticide Act (FIFRA) predominantly focuses on the component which would serve as a pesticide, in this case, the tetracycline Trans- Activator Variant (tTAV) protein that Oxitec's GE mosquitoes have been genetically engineered to express. However, it is critical that the EPA examine the whole mosquito, the method of delivery in this case, and its direct and indirect impacts on the environment, human and animal health." (Friends of the Earth 0342 p. 5)

The Center for Food Safety (0344) and GeneWatch UK (0335) stated that:

> "EPA cannot adequately protect human and animal health and the environment by simply focusing the assessment of risks on the active ingredient tTAV–OX5034 (the genetic sequence which provides the genetically engineered killing mechanism for the mosquitoes). This is because other introduced traits, which are present due to the use of a non-native strain of mosquito (such as altered disease transmission properties), may also pose serious risks to human and animal health and the environment." (Center for Food Safety 0344 p. 2; GeneWatch UK 0335 p. 2)

GeneWatch UK (0335) stated that:

> "Regulatory actions under the Insecticide, Fungicide and Rodenticide Act (FIFRA) focus largely on the active ingredient (intended to act as a pesticide by killing pests), namely the tetracycline Trans- Activator Variant (tTAV) protein that Oxitec's GE mosquitoes have been genetically engineered to express. However, in this case, Oxitec is not releasing an inert ingredient but a living organism. Thus, not only the active ingredient, but also its method of delivery must be carefully considered." (GeneWatch UK 0335 p. 3)

H. Comments Arguing That EPA Should Ensure Post-Release Control Measures

Five comments (0027, 0052, 0076, 0121, 0173, 0182, 0315) were received on post-release control measures. Most of these comments revolve around requests for a mitigation strategy to be in place in the event OX5034 does not behave as anticipated. One comment in this group indicated that a post-testing review period should be instituted.

Anonymous (0173) stated that should the EUP be granted and testing proceeds:

> " If allowed to be used, there must be 2 year review period whereas all interested parties must submit proof to support their position and gauge it's impact . . . ." (Anonymous 0173 p.1)

Commenter S.C. Ray (0027) commenting on the Evans et al article, stated that:

> " . . . if this represents a failure of design it needs a coherent mitigation strategy to avoid potentially deleterious and unchecked changes to the genetic makeup of domestic mosquito populations." (S.C. Ray 0027 p. 1)

H. Scott (0052) stated that:

> "These experimental, genetically modified mosquitoes may pose a real risk to human and animal health and safety that may not be currently understood. The anticipation is that these mosquitoes will not be able to reproduce or bite (and thus transmit disease), these claims are NOT proven or guaranteed, and there exists no effective plan to contain or eradicate ALL OX5034 mosquitoes if this were to happen." (H. Scott 0052 p. 1) [Emphasis in the original]

Anonymous (0182) raised the question:

> "What if there is a mutated gene sequence and we have GMO mosquito that could cause greater damage to people. If this happens do you have the funds to clean up the mess, much like BP and the spill off the coast in Texas?" (Anonymous 0182 p. 1)

Commenter Hasham (0315) questioned:

> "Can these genetically modified Mosquitoes be killed later if needed. . . . Who will be held accountable if the results comes [sic] out in a disaster?" (Hasham 0315 p. 1)

On the other hand, some commenters (e.g., 0121) argued that:

> "There will be no way to contain them once released if there is an issue." (Anonymous 0121 p. 1)

I. Comments Arguing That EPA Must Address Ethical Concerns

Comments on ethical concerns revolve around three topics; (1) whether humans present in the EUP testing area should be considered human subjects and the testing be subject to the requirements of human studies rules; (2) OX5034 might be useful to communities with a limited tax base and thus be an environmental justice consideration; and (3) sacredness of the natural world and its preservation.

1. Human Subjects and Informed Consent

Forty-five comments questioning whether humans in the test area should be considered research subject were received. (0014, 0031, 0038, 0058, 0088, 0092, 0096, 0097, 0101, 0106, 0113, 0118, 0119, 0128, 0129, 0134, 0135, 0136, 0148, 0171, 0184, 0189, 0193, 0204, 0206, 0213, 0214, 0228, 0237, 0245, 0275, 0284, 0285, 0287, 0296, 0300, 0306, 0317, 0318, 0323, 0329, 0334, 0335, 0342, 0344). Most of these comments simply objected to the testing on the basis of fears of being exposed to OX5034 mosquitoes. These commenters simply indicated that they do not consent to being part of any testing or stated their belief that informed consent is required for the proposed testing, or voiced concerns that they would be subjects in experiments that they do not support. These commenters do not provide any detailed rationale for the commenters' position. Other comments are more specific. These comments argued that informed consent has to be part of any testing of OX5034 because: (1) OX5034 mosquitoes might interact with humans in the test area; (2) limitations on the use of other methods of mosquito control might affect humans in the test area; and (3) long-term effects of potential interactions between OX5034 and humans have not been studied. Examples of the types of comments received are provided below.

GeneWatch UK (0335) and Center for Food Safety (0344) stated that:

> "We note that, were an experimental use licence to be granted, the requirements of EPA's human studies rule (40 CFR Part 26) should be followed, due to the exposure of human subjects (including children) to the proposed open releases of GE mosquitoes, and the potential limitations on the use of other methods of mosquito control that may need to be applied during the experiments." (GeneWatch UK 0335 p. 13; Center for Food Safety 0344 p. 16)

Commenter D. Rubin (0317) stated that:

> "This is a biological experiment. The mosquitoes will interact with humans in our environment and no one knows all of the implications of that interaction." (D. Rubin 0317 p.1)

Friends of the Earth (0342) stated that:

> "The release of GE mosquitoes as an attempt to curb the spread of disease should be considered a medical trial and must follow the laws and guidelines in place to protect human subjects in medical trials. Central to ethics on human subject trials is the idea of free and informed consent. . . . It is critical that communities, and in particular, the communities that would be on the front line of this experiment, give consent to being part of this experiment. . . Community members must be informed throughout the process through a number of mechanisms, including the establishment of local institutional review boards and ethics committees and hosting of community meetings and public forums. Community members must know the parameters of the trial areas, have a right to leave the field trial areas or demand the halt of the experiment entirely if they so decide." (Friends of the Earth 0342 p. 6) [Footnote omitted]

Commenter M. Daly (0300) contended that in the absence of an Informed Consent process Oxitec had "not obtained informed consent to Legally run this trial." She stated that:

> "Informed Consent is a voluntary agreement to participate in research. It is not merely a form that is signed but is a process, in which the subject has an understanding of the research and its risks. Informed consent is essential before enrolling a participant and ongoing once enrolled. Final Rule revisions of the Common Rule (https://www.hhs.gov/ohrp/regulations-and-policy/regulations/finalized-revisions-common-rule/index.html)." (M. Daly 0300 p. 1)

Commenter M. Daly (0300) pointed out that the Common Rule defines a human research subject as:

> " . . . , an individual whose interests may be compromised as a result of interventions in a research study." (M. Daly 0300 p. 1)

Commenter M. Daly (0300) went on to explain that "interventions" refers to:

> " . . . both to the experimental procedure being investigated as well as to non-experimental data collection procedures. More specifically, a human research subject is an individual: 1) who is directly intervened upon by an investigator as either (a) a recipient of a study intervention or (b) as someone who undergoes non-experimental interventions to collect data; 2) who is deliberately intervened upon via manipulation of the individuals environment by the investigator in such a way as to have a direct effect on the individual; 3) who communicates or has interpersonal contact with an investigator for the purpose of collecting data through, for example, interviews, focus groups, or questionnaires; and 4) about whom an investigator obtains identifiable private information for the purpose of collecting data. . . ." (M. Daly 0300 p. 1)

Commenter M. Daly (0300) contended that people in the test area were human research subjects:

> "When their home or property is accessed and the location recorded as a spatial variable for the release or collection of mosquitoes because the precise location of the household is important for entomological reasons and these data constitute identifiable private information at the household." (M. Daly 0300 p. 1)

Commenter M. Daly (0300) went on to state that:

> "I am writing this today to let you know that the process of Informed Consent, which is required by law, was not taken into consideration. Oxitec has never released any safety data in regards to human health during a trial. Based upon their theories they claim it is not necessary." (M. Daly 0300 p. 1)

Commenter M. Daly (0300) added that while a referendum was held in Key Haven on the acceptability of Oxitec testing in the area:

> "There is a distinct difference between a generic vote of uninformed citizens and informed consent." (M. Daly 0300 p. 1)

With regard to the referendum, M. Daly (0300) stated the belief that:

> "A small group of residents believed that the people of the original trial area in Key Haven were properly informed of both sides of this issue and requested that a

> referendum be added to our local elections and leave it to the people to decide. The FKMCD decided at the last minute to add a Keys wide referendum to the ballot also. The residents of the rest of the Keys were not informed about much other than the Oxitec radio ads running all day leading up to the vote. Key Haven voted against it. The uninformed Keys wide voters passed it by a slight margin." (M. Daly 0300 p. 1)

Anonymous (0237), stating opposition to the testing, indicated that the belief that the federal government did not have the:

> " . . . right to use the residents of Florida and Texas as guinea pigs for an untested hypotheses which might have unknown health ramifications in the future. The majority of these residents aren't even aware of this proposed plan and accepting public comments isn't the equivalent of earning the informed consent of the people." (Anonymous 0237 p. 1)

Commenter C. Tong (0106) stated that:

> "Florida citizens are not guinea pigs for this companies [sic] services. Letting rogue genetics into our mosquito populations demands more time for education and Voting." (C. Tong 0106 p. 1)

Anonymous (0148) stated that:

> "OXITEC has admitted that an unknown number of biting females will be released, but no one has conducted clinical tests on consenting, informed, adult humans bitten by these females." (Anonymous 0148 p. 1)[Emphasis in the original]

Anonymous (0148) added that:

> "Experiments with genetically modified organisms should only be conducted in a controlled environment with consenting informed adults, and should never be allowed into the public domain before these clinical and biological tests are conducted." (Anonymous 0148 p. 1)

Anonymous (0204) stated that:

> "Releasing Oxitecs [sic] genetically modified mosquitoes goes directly against the Nuremberg Code. Oxitec does NOT have permission to experiment on me." (Anonymous 0204 p. 1)

Along the same lines, Anonymous (0296) stated that because the long-term effects have not been studied:

> " . . . , the release of GM mosquitoes without the informed consent of every individual affected constitutes a human rights violation. This would leave the city or county releasing GM mosquitoes liable. According to the Nuremberg Code: The voluntary consent of the human subject is absolutely essential." (Anonymous 0296 p. 1)

Anonymous (0287) stated that:

> "THIS IS DANGEROUS THE PUBLIC AND IS A VIOLATION OF THE NUREMBERG CODE. ....which states: The voluntary consent of the human subject is absolutely essential. .....Without informed consent of EVERY individual who could be affected YOUR LOOKING AT HUMAN RIGHTS VIOLATION." (Anonymous 0287 p. 1) [Emphasis in the original]

Commenter D. Rubin (0317) stated that:

> "As a resident of Florida who will be affected by this experiment and does not consent to being a part of it, I urge the EPA to reject the application of Oxitec, Ltd. who request an experimental use permit (EUP) for the OX5034 *Aedes aegypti* mosquitoes expressing tetracycline Trans-Activator Variant (tTAV-OX5034) protein (identified by number 93167-EUP-E). I repeat, I do not consent to being a part of this experiment and if you allow the release of the gmo mosquitoes, I will be forced against my will to be part of a human experiment along with all of the children who, if we were war criminals, would be protected from such experimentation under the Geneva Convention, Rule 92. Mutilation and Medical, Scientific or Biological Experiments." (D. Rubin 0317 p.1)

Commenter D. Rubin (0317) stated that:

> "They may also modify our environment in a ways we can not possibly foresee, causing far more harm than good. These open-ended experiments in our environment are foolish to say the least, criminal to say the most. What gives EPA or Oxitec the right to tinker with our state--and planet--when EPA can give no guarantee of safety. Can EPA give a guarantee to the people of Florida?" (D. Rubin 0317 p.1)

Commenter J. Birk (0097) stated that:

> "It is illegal to experiment on humans in United States, without their signed consent. These GMO mosquitoes are nothing more than that, an experiment. No one really

knows what the long term consequences to the population, livestock, and nature will be." (J. Birk 0097 p.1)

Anonymous 0184 stated that:

> "If this so-called "test" goes unexpectedly, there is no sure way to undo the damage done. Scientists cannot really know how this will negatively effect [sic] other plants, animals, and possibly humans. There is no way to opt out of a test that involves the environment around us on such a scale, so such a test is, in-fact, immoral. The Nuremberg Code states that people must have informed consent when it comes to experimentation, as well as the ability to opt out. Performing these tests give neither to the people in the areas that will or may become effected by this experiment." (Anonymous 0184 p. 1)

2. Environmental Justice

One commenter, J. M. Conlon, American Mosquito Control Association, (0263), suggested OX5034 might be useful to communities with a limited tax base and thus be an environmental justice consideration. He stated that:

> "OX5034 *Aedes aegypti* mosquitoes are particularly well-suited to suppress vector mosquito populations below disease transmission threshold in smaller, rural communities not possessing the tax base to establish and maintain fully resourced county/municipal mosquito control programs. This is an issue of environmental justice that could be addressed, in large part, by the utilization of OX5034 mosquitoes as control measures." (J. M. Conlon, American Mosquito Control Association, 0263 p. 2)

3. Preservation of the Natural World

Some commenters raised issues revolving around preservation of the natural world. These included; (1) ethical concerns about implementing any strategy that might result in the elimination of a species; (2) the appropriateness at this time of attempting to reduce any insect population in light of the documented decline in insect populations world-wide; and (3) opposition to genetic engineering on the belief that a genetically engineered organism is unnatural. (0008, 0009, 0015, 0039, 0051, 0058, 0061, 0067, 0069, 0070, 0077, 0099, 0105, 0115, 0118, 0126, 0129, 0142, 0157, 0158, 0172, 0187, 0195, 0197, 0181, 0187, 0281, 0316). Examples of these types of comments are listed below.

Anonymous (0008) argued that:

> “My foremost concern is the unforeseen repercussions of trying to eradicate a species, especially in this age of many unintended extinctions. Using the natural world as a testing ground for such gene alteration of a particular species is unconscionable and unethical.” (Anonymous 0008 p. 1)

Anonymous (0009) stated that:

> “This method of making the mosquito population sterile and unable to reproduce may be a bad idea in light of the recent research indicating there has been a decline in overall insect populations all over the world. Research conducted in Germany's nature reserves have found that they have a 76% reduction in insect populations in the last 3 years. I don't think we should be devising plans to further reduce insect populations at this time.” (Anonymous 0009 p.1)

Anonymous (0181) stated that:

> “Don’t mess with genetics through GMO anything. Messing with genetics is messing with Gods laws and their sacredness which should be preserved and not GMOed.” (Anonymous 0181 p. 1)

Anonymous (0069) stated that:

> “Please do the right thing this time and don't allow genetically modified mosquitoes or anything to be continued. Genetically modified food, insects or anything is unnatural and anything unnatural will eventually cause problems for everyone on this planet and already has! This is common sense.” (Anonymous 0069 p. 1)

Commenter S.L. Smith (0099) stated that:

> “None of those working on this project is GOD ALMIGHTY, or has His skill in creating creatures; indeed, they're playing in the Devil's workshop and the results thereof can only be harmful! It's not for us to play with the DNA coding of anyone or anything. You've been covering up and lying about the damage done by GMO foods for over a decade now, . . .” (S.L. Smith 0099 p. 1) [Emphasis in the original]

Anonymous (0172) stated that:

> “Please do not release modified mosquitoes. We do not need to add some more to our environment. The eco system is designed by a master planner. We have no idea what or how this will effect us or the environment and once they are released there is no taking

them back. It is bad enough that our food was modified." (Anonymous 0172 p. 1) [Typographical error in the original]

Commenter K. Bell (0126) requested "please leave Mother Nature alone" adding that:

> "As someone whose life has been adversely affected by Lyme Disease this is beyond terrifying! Do you all know that mosquitos suck your blood and can and do infect people with Lyme? Is this population control?" (K. Bell 0126 p. 1)

Anonymous (0281) stated that:

> "This is how we ended up with Lyme disease. Stop playing God. No more GMO animals. Especially disease carrying animals." (Anonymous 0281 p. 1)

Anonymous (0058) stated that:

> "You also assume zero liability for any harm done by this Godless experiment that can and most likely will go wrong in a multitude of ways. You have already allowed the weaponization of ticks and now you're looking to do the same thing with mosquitoes. Why can't you ever leave nature alone? In your arrogance, you think that you can manipulate nature and make your own synthetic version." (Anonymous 0058 p. 1)

Anonymous (0187) stated that:

> "Finally, when we alter the environment and ecosystem with manmade organisms, we inevitably alter the susceptibility of the environment." (Anonymous 0187 p. 1)

4. Comments Urging the Agency to Proceed Cautiously

One commenter, M. Wilcox (0129), urged that:

> "Please utilize the precautionary principle and avoid what has happened in Brazil on our own soil!" (M. Wilcox 0129 p. 1)

J. Comments Arguing EPA Must Prepare a Full EIS Under the National Environmental Policy Act

Some commenters (0038, 0320, 0342, 0335, 0344) argued that EPA must prepare a full Environmental Impact Statement (EIS) under the National Environmental Policy Act (NEPA). Some comments explained why the commenters believed NEPA was an appropriate vehicle for

evaluating OX5034. Other comments cited a number of scientific questions that the commenters believed would be addressed better in an analysis under NEPA than an assessment under FIFRA.

1. Comments Explaining Why the Commenter Believed NEPA was an Appropriate Vehicle for Evaluating OX5034

Some commenters (0335, 0342, 0344) stated why they believed NEPA was a more appropriate vehicle for assessing OX5034 than FIFRA.

Friends of the Earth (0342) stated that:

> "Although in some cases proposed actions under FIFRA have been exempt from NEPA, Oxitec's proposed actions for a deliberate release of disease vectors into the environment raise complex environmental issues which may not be adequately captured under FIFRA, therefore an assessment under NEPA should also be required. This assessment should include a full EIS and comparison with the alternatives that do not involve the same risks." (Friends of the Earth 0342 p. 6)

Friends of the Earth (0342) added that:

> "Under the National Environmental Policy Act (NEPA), the EPA should consider all environmental effects of the environmental release of Oxitec's GE mosquitoes, analyze potential environmental effects, and analyze alternatives to these actions, effects of proposed actions, and analyze how to restore and enhance environmental quality to the extent practicable. As part of these requirements, the EPA should undertake a full EIS so that it may fully examine the potentially substantial impacts that the proposed action may have." (Friends of the Earth 0342 p. 6)

The Center for Food Safety (0344) stated that:

> "A full EIS should be prepared under the National Environmental Policy Act (NEPA), and this should be subject to further consultation. The EIS should include consideration of the EPA's responsibilities under other environmental legislation, including the Endangered Species Act." (Center for Food Safety 0344 p.4)

The Center for Food Safety (0344) and GeneWatch UK (0335) explained that:

> "The National Environmental Policy Act (NEPA), 42 U.S.C. §4321 et seq., as implemented by the Council on Environmental Quality (CEQ) Regulations (40 CFR Parts 1500 through 1508), requires that Federal agencies include in their decision-making processes

appropriate and careful consideration of all environmental effects of proposed actions, review all potential environmental effects of proposed actions and their alternatives for public understanding and scrutiny, avoid or minimize adverse effects of proposed actions, and restore and enhance environmental quality to the extent practicable (40 CFR §6.100). The EPA shall integrate these NEPA requirements as early in the Agency planning processes as possible. The environmental review process shall be the focal point to ensure NEPA considerations are taken into account. This is the process used to comply with section 102(2) of NEPA or the CEQ Regulations including development, supplementation, adoption, and revision of NEPA documents." (Center for Food Safety 0344 p.5: GeneWatch UK 0335 p. 3-4)

GeneWatch UK (0335) stated that:

> "As part of these requirements, the EPA must undertake an environmental review and prepare either an Environmental Assessment (EA) and Finding of No Significant Impact (FONSI) or an Environmental Impact Statement (EIS) and record of decision (ROD) for the proposed action. Consistent with 40 CFR 1500.5(g) and 1502.25, the Responsible Official must determine the applicability of other environmental laws and executive orders, to the fullest extent possible (40 CFR §6.201). This is likely to include, for example, the Endangered Species Act, so that the risks to threatened and endangered species (for example, through consumption of the GE mosquitoes) can be assessed. Public participation requirements are outlined in 40 CFR §6.203, including requirements for public consultation." (GeneWatch UK 0335 p. 4)

The Center for Food Safety (0344) and GeneWatch UK (0335) argued that EPA should prepare an EIS for OX5034 prior to making an EUP determination because:

> "Actions under FIFRA have traditionally been exempt from NEPA, but this depends on whether the assessment under FIFRA is functionally equivalent to the assessment under NEPA, ensuring full and adequate consideration of environmental issues. It is not a broad exemption but a "*narrow exemption from the literal requirements for those actions which are undertaken pursuant to sufficient safeguards so that the purpose and policies behind NEPA will necessarily be fulfilled*". Although this exemption may apply for traditional applications of chemical and biochemical pesticides, there are many issues associated with the release of GE mosquitoes into the environment which may not be adequately captured by assessment under FIFRA . . . . Therefore an assessment under NEPA is also required." (Center for Food Safety 0344 p.5-6; GeneWatch UK 0335 p. 4) [Emphasis in the original] [Footnote omitted]

2. Comments Citing Examples of Scientific Questions the Commenter Believes Can Be Better Assessed Under NEPA

Some commenter (0335, 0342, 0344) offered examples to support their opinion that NEPA is a better vehicle to address scientific questions associated with OX5034.

The Center for Food Safety (0344) and GeneWatch UK (0335) furthered their argument that NEPA was a more appropriate vehicle than FIFRA by stating that:

> "The issues covered by the EA or EIS are likely to be broader than those considered under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), because regulatory actions under FIFRA focus largely on the active ingredient (intended to act as a pesticide by killing pests), namely the tetracycline Trans-Activator Variant (tTAV) protein that Oxitec's GE mosquitoes have been genetically engineered to express. However, in this case, Oxitec is not releasing an inert ingredient but a living organism. This organism is a pest and human disease vector . . . , and a genetically engineered (GE) organism (regulated as a plant pest under 7 CFR part 340). It is also an organism which may introduce or disseminate a contagious or infectious disease of animals (regulated under 9 CFR part 122): relevant diseases include dog heartworm, *Dirofilaria immitis*; lumpy skin disease virus; myxoma virus; fibroma virus; and Rift Valley Fever, as well as human diseases such as dengue which may also infect primates and perhaps dogs. Thus, not only the active ingredient, but also its method of delivery, and the impact on the environment and human and animal health of associated complex changes in ecology, must be carefully assessed in a manner which ensures compliance with all relevant regulations and protects human and animal health and the environment. This method of delivery of the active ingredient introduces additional concerns and potential adverse impacts on the environment and human health . . . ." (Center for Food Safety 0344 p. 6; GeneWatch UK 0335 p.4-5) [Footnotes omitted]

The Center for Food Safety (0344) and GeneWatch UK (0335) argued that:

> ". . . , the EPA should prepare an EIS, as the potential impacts of the proposed action are complex and significant and as the countries major environmental review agency, EPA should be expected to do a fully adequate review of the environmental effects of this new mosquito that likely will persist in the environments of the Florida Keys and Harris County, Texas." (Center for Food Safety 0344 p. 5; GeneWatch UK 0335 p. 6)

The Center for Food Safety (0344) and GeneWatch UK (0335) concluded by stating that:

> ". . . , the movement of insects, mites and ticks that affect man or vector human diseases require permits from the CDC, and it is unclear whether open release of such organisms, especially if biting females are included and/or non-native strains are used, can be permitted. It is unclear why taking this risk would be justified, in comparison with alternatives, and at the very least a full assessment of this risk must be made by publishing a full EIS for consultation under NEPA, before the proposed experimental

releases of GE mosquitoes are undertaken. The EIS must include data to quantify the effectiveness of the female-killing mechanism engineered into the OX5034 strain, rather than relying on Oxitec's claim that it is 100% effective." (Center for Food Safety 0344 p. 7; GeneWatch UK 0335 p. 6)

GeneWatch UK (0335) stated that:

> "Increases in non-target mosquito species as a result of the proposed releases could pose risks to human and animal health, as could increases in the target species in areas neighbouring the releases. Complex ecosystem responses could result from altered mosquito population dynamics and wildlife may be affected by ingestion of the GE mosquitoes. These risks must be assessed in a full EIS, including potential risks to relevant species under the Endangered Species Act." (GeneWatch UK 0335 p. 11)

Friends of the Earth (0342) stated that:

> " . . , the application doesn't include an environmental assessment (EA) or environmental impact statement (EIS). While we support the EPA's intentions to limit mosquito populations and the spread of mosquito borne disease, Friends of the Earth believes this experiment with Oxitec's OX5034 mosquitoes is too risky for Florida and Texas' ecosystems and public health and is fraught with many unanswered and critical questions that Oxitec needs to answer for the EPA and the public prior to an approval for release." (Friends of the Earth 344 p. 1)

GeneWatch UK (0335) stated that:

> "The potential spread of antibiotic resistance could pose a serious risk to human and animal health. It is therefore essential . . . to assess the risks to human health and the environment in a full EIS." (GeneWatch UK 0335 p. 9)

Friends of the Earth (0342) stated that:

> "There should be a full Environmental Impact Statement (EIS) under the National Environmental Policy Act (NEPA), . . ." (Friends of the Earth 0342 p.2)

Friends of the Earth (0342) stated that:

> "In light of the unanswered questions and the gaps in data analysis, Friends of the Earth urges EPA to reject Oxitec's application for genetically engineered mosquitoes and if the EPA will not conduct a full EIS, Oxitec should provide information that would be

equivalent to a full Environmental Impact Statement (EIS)." (Friends of the Earth 0342 p. 7)

Commenter K. Gould (0320) called on the EPA to:

> " . . . delay the experiment and conduct a thorough literature review of all related studies detailing the approach in this proposal." (K. Gould 0320 p. 1)

K. Comments Stating that EPA Must Comply with the Endangered Species Act

Some commenters (0335, 0342, 0344) argued that EPA must comply with the Endangered Species Act (ESA). For example,

The Center for Food Safety (0344) stated that:

> "Oxitec's compliance with all other relevant laws must be considered, including those covering the deliberate release of disease vectors." (Center for Food Safety 0344 p. 6)

Friends of the Earth (0342) stated that:

> "In addition to preparing a full EIS for public consideration, the EPA should ensure that it is complying with the Endangered Species Act." (Friends of the Earth 0342 p. 6)

L. Comments Stating that EPA Must Comply with the Veterinary Feed Directive

Some commenters (0293, 0334, 0335, 0344) argued that EPA must comply with the Veterinary Feed Directive, a directive issued by the U.S. Food and Drug Administration that addresses the use of antibiotics in animal feed[13], as well as being consistent with the World Health Organization Global Action Plan on Antimicrobial Resistance.

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "The potential spread of antibiotic resistance could pose a serious risk to human and animal health. It is therefore essential to consider whether Oxitec's use of antibiotics is lawful under the Veterinary Feed Directive (21 U.S.C. §354) and any other relevant legislation or executive orders, . . ." (GeneWatch UK 0335 p. 9; Center for Food Safety 0344 p. 11)

[13] https://www.fda.gov/animal-veterinary/development-approval-process/veterinary-feed-directive-vfd

Similarly, J.W. Norris (0334) stated that:

> "Investigation into the OX513A's potential to spread antimicrobial resistance is further supported WHO's 'Global Action Plan on Antimicrobial Resistance.' Outcome four of strategic objective two speaks specifically to the need to monitor antibiotic use in animal production. Although it does not directly speak to this issue, the concept of mass producing – and distribution of – antibiotic dependent insects on a global scale is a novel issue made possible only by recent technological advances." (J.W. Norris 0334 p. 3 of the Attachment) [Footnotes omitted]

Commenter J.W. Norris (0293) further stated that use of tetracycline for production of OX5034:

> " . . . conflicts directly with World Health Organization's stated policy: 'Not use antibiotics for growth promotion or to prevent diseases in healthy animals'." (J.W. Norris 0293 p.1)

M. Comments Questioning Whether Oxitec Can Be Released from the Contained Use Requirements of an Import Permit

Three commenters (0335, 0342, 0344) questioned whether, given regulations at 42 CFR 71.54, open release of OX5034 can lawfully be permitted under an EUP issued under FIFRA.

GeneWatch UK (0335) and the Center for Food Safety (0344) stated that:

> "The *Aedes aegypti* mosquito that Oxitec proposes to release is itself categorised as a pest, under 7 U.S.C. § 136(t) and § 136w(c)(1), because this mosquito species may be injurious to health or the environment. Mosquitoes are listed as Pests of Significant Public Health Importance. Further, the *Aedes aegypti* mosquito is a disease vector, as defined in 42 CFR §71.54: "*Any animals (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal products (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are known to transfer or are capable of transferring an infectious biological agent to a human*". The movement of human disease vectors requires permits from the Centers for Disease Control (CDC). Import permits are granted under import regulations for infectious biological agents, infectious substances, and vectors (42 CFR §71.54) and the importer is required to remain in compliance with all of the permit requirements and conditions that are outlined in the permit issued by the CDC, which would not normally allow any open release of such disease vectors into the environment. A permit issued under this part is not required under certain circumstances, but these do not include the issuing of a licence for experimental use, or full product approval, of a pesticide under FIFRA (although an FDA licence as a New Animal Drug – the previous regulatory process - does allow exemption from a CDC permit). It is therefore unclear whether open release of such organisms can

be lawfully permitted through the proposed mechanism of granting an experimental use permit under FIFRA. Particular concerns arise in this regard because of the potential release of biting females . . . and the use of a non-native imported strain of the *Aedes aegypti* mosquito . . , which is expected to lead to non-native hybrid mosquito strains becoming established in the environment. The EPA must therefore clarify the legal basis under which it proposes that Oxitec should be released from the contained use requirements of its import permit, in order to allow its GE mosquitoes to be deliberately released into the environment." (Center for Food Safety 0344 p.4; GeneWatch UK 0335 p. 3) [Footnotes omitted]

Friends of the Earth (0342) stated that:

> "Also, because the *Aedes aegypti* mosquito is considered a disease vector, the EPA should clarify the legal basis for a proposal which would allow Oxitec to be released from the contained use requirements of its import permit, as delineated by the Center for Disease Control, in order to allow its GE mosquitoes to be deliberately released into the environment. As articulated in the GeneWatch UK public comments, it is unclear whether open release of GE mosquitoes can be lawfully permitted through the proposed mechanism of granting an experimental use permit under FIFRA." (Friends of the Earth 0342 p. 5) [Footnote omitted]

N. EPA Should Not Rely on the Registrant Provided Assessment Data/Information

Several commenters (0038, 0089, 0295, 0296, 0316, 0331) argued that EPA should not rely on registrant provided data/information.

The Florida Keys Environmental Coalition (0331) argued that based on past experience with Oxitec, Ltd.'s attempt to gain community acceptance for their products in the Florida Keys:

> "Oxitec has a long and conflicted relationship with recognizing the flaws in their own system performance." (Florida Keys Environmental Coalition 0331 p. 1-2)

The Florida Keys Environmental Coalition (0331) stated that:

> "Trusting vendors for product evaluation and description cannot be anymore relevant when looking at the Boeing 737 Max where the FAA ran a similar risky program. The EPA should place its mission at risk by permitting novel complex science to be evaluated from a vendor written Environmental Assessment (EA)." (Florida Keys Environmental Coalition 0331 p. 3)

J. Barton (0295) echoed this comment, stating that:

> "Our government agencies must not rely only on data from the companies that would profit from genetically engineered organisms to decide what information the public and regulators should know." (J. Barton 0295 p. 2)

Commenter J. Butler (0089), referring to the Evans et al paper, stated that:

> "Oxitecs [sic] claims about its GM mosquitoes cannot be trusted and should not be considered reliable based on the results of this previous experience, with this release of GM mosquitoes in Brazil" (J. Butler 0089 p. 1)

EPA Response to Unit XII.A. – Comments that EPA Should Extend the Comment Period. The amount of information EPA could share with the public at the time the Agency received requests for an extension of the comment period was limited as EPA had not yet performed its analysis of the submission. As the major thrust of the requests for an extension were generally coupled with a request for additional information/data, EPA concluded that an extension of the comment period in the absence of additional data/information was unlikely to meet the commenters requests. Therefore, EPA did not extend the comment period. However, EPA is now including in the docket created for the OX5034 submission its analysis of the submission as well as this Response to Comment document to make information available to the public. Included in the docket are documents detailing EPA's analysis of the submission. The documents detailing EPA's analysis of the submission are the Human Health and Environmental Risk Assessment, the Review of Section G, and the Memorandum on Vectorial Capacity that can be found in the docket (EPA-HQ-OPP-2019-0274).

EPA Response to Unit XII.B. – Comments that EPA Should Offer an Additional Opportunity for Public Comment. EPA's public participation policy where-in EPA offers an additional opportunity for comment after completing its analyses but prior to making a regulatory decision applies to registration actions rather than to EUP requests[14]. However, EPA is including in the docket created for this application its analysis of the data as well as this Response to Comments document to allow the public to examine the Agency's approach to the OX5034 request for an EUP. The documents detailing EPA's analysis of the EUP submission are the Human Health and Environmental Risk Assessment, the Review of Section G, and the Memorandum on Vectorial Capacity that can be found in the docket (EPA-HQ-OPP-2019-0274). Should the company apply for a registration for OX5034, information in this docket will be part of the record and available to the public.

EPA Response to Unit XII.C. – Comments that EPA Should Ensure Transparency. EPA recognizes the value of transparency in its regulatory actions and is committed to taking public comment into account in its decision making. Some of the commenters requesting greater transparency requested that EPA's in-depth technical analysis of the OX5034 request for an EUP

[14] https://www.epa.gov/pesticides/pesticide-program-public-involvement-opportunities

be made available to the public. EPA is including its analysis of the application for an EUP to permit limited testing of OX5034 in the docket created for this application, i.e., the Review of Section G, the Human Health and Environmental Risk Assessment and the Memorandum on Vectorial Capacity. EPA is also including this Response to Comments document in the docket.

EPA Response to Unit XII.D. – Comments that EPA Should Seek Advice From Independent Committees of Experts. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) as described in FIFRA section 25(d) provides independent scientific advice to the EPA on health and safety issues related to pesticides. In general, SAP advice is sought for products where wider distribution in the environment, e.g., such as that frequently observed with a registered product, than presented by a product being tested under an EUP. EPA has, however, for this EUP, sought the advice of technical experts at the US Centers for Disease Control and Prevention (CDC) on several of the technical issues presented by OX5034 release, including their analysis of the recent publication by Evans et al. The CDC advice has been incorporated into the decision reached by the Agency on the request for an EUP to field test OX5034 mosquito.

EPA Response to Unit XII.E. Comments Arguing that EPA Should Seek Public Input.
Referendums – known for example as "ballot measures, propositions or simply questions" – allow citizens to vote directly on an issue at the ballot box. EPA has no authority to request or to institute referendums on issues. Rather, the authority to call or hold referendums is the purview of state and local governmental authorities in a particular voting district.

With regard to comments requesting that EPA hold public meetings, the EUP regulations regarding "Publication" at 40 CFR 172.11 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….
>
> (b) Public hearing. The Administrator *may* hold a public hearing … when he determines that there is sufficient interest in the application to warrant a hearing, based upon the comments received in response to the Notice of Receipt of an Application, or that a hearing would otherwise be in the public interest.

40 CFR 172.11 (emphasis added). While the EPA "shall" publish a NOR in the Federal Register upon a finding that issuance of the EUP may be of regional or national significance, the Agency "may" hold a public hearing when EPA determines that a public hearing is warranted. Here, EPA published a Notice of Receipt (NOR) of the EUP application in the Federal Register, 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 EPA believes that the NOR and

public comment period already provided fulfill the requirements of the "publication" regulations, and the Agency does not intend at this time to exercise its discretion to hold a public hearing.

EPA Response to Unit XII.F and Unit XII.G. Comments Challenging EPA's Interpretation That FIFRA Can Be Used to Regulate OX5034 Mosquito. Comments Arguing That EPA Needs to Develop New Regulations for GE Insects. EPA is primarily regulating tTAV-OX5034 and DsRed2-OX5034, much like EPA regulates "plant-incorporated protectants" (PIPs), defined in part as "a pesticidal substance that is intended to be produced and used in a living plant, … and the genetic material necessary for production of such a pesticidal substance" (40 CFR 174.3). EPA has jurisdiction to regulate these substances under FIFRA because tTAV-OX5034 is intended for preventing, destroying or mitigating a pest, and therefore meets the definition of "pesticide" under Section 2(u) of the FIFRA.

In 2010, the U.S. Food and Drug Administration (FDA) announced it would regulate intentionally altered genomic DNA as a "new animal drug" (NAD) under the Federal Food, Drug, and Cosmetic Act (FFDCA). NADs are explicitly excluded from the definition of "pesticides" in section 2(u) of FIFRA, which states, in part, that "… the term 'pesticide' shall not include any article that is a 'new animal drug' within the meaning of section 321(w) of Title 21…." However, in October 2017, FDA issued "Guidance for Industry" (GFI) #236, in which FDA states that it does not view intentionally altered genomic DNA that has been genetically engineered into mosquitoes for population control purposes as "NADs," and since tTAV-OX5034 otherwise meets the definition of a "pesticide" under FIFRA, tTAV-OX5034 engineered into mosquitoes is regulated by EPA as a pesticide.

Regulation of tTAV-OX5034 engineered into mosquitoes designed for population control is simply the regulation of a pesticide, which EPA believes is fully covered by existing FIFRA regulations. As a result, EPA does not agree that new regulations are necessary in order to grant the present EUP upon which comment has been taken.

EPA has sufficient authority under FIFRA to ensure that no unreasonable adverse effects on health and the environment occur during EUP testing even in the absence of regulations directed specifically at GE insects.

EPA Response to Unit XII.H. – Comment that EPA Should Ensure Post-Release Control Measures. With regard to the comment that there should be a 2-year review period post-testing, the regulatory system established by FIFRA requires the Agency to review data submitted to support a registration request. That registration request would include data developed during testing under an EUP. Under FIFRA, EPA may register (*i.e.*, authorize an entity to sell or distribute a pesticide product with particular conditions of use) a pesticide when it will not cause unreasonable adverse effects to man or the environment. To support an application for a registration, EPA requires extensive scientific data and information on the potential health

and environmental effects of a pesticide. The data and information allow EPA to evaluate the potential of the pesticide to harm nontarget organisms, including humans, wildlife, and plants. EPA reviews these data and information and establishes appropriate use conditions to ensure that use of the pesticide will not cause unreasonable adverse effects to man or the environment. The Agency also can request public comment on the product proposed for registration, and this presents an opportunity for the public to provide the Agency additional information.

With regard to comments requesting that a mitigation strategy be in place prior to testing, in order to issue the EUP, EPA must ensure that OX5034 will not cause unreasonable adverse effects to man or the environment. Based on its analysis, EPA believes the probability of OX5034 presenting a problem requiring mitigation measures is very low. The self-limiting function of the tTAV-OX5034 gene ensures the gene does not remain in the *Ae. aegypti* mosquito population (see Unit II.C.2 "Persistence of the OX5034 transgene in the environment post-release" in the Human Health and Environmental Risk Assessment in this docket). However, Oxitec is required to have a mitigation plan in place should an unanticipated problem arise. The OX5034 mosquito is sensitive to chemical pesticides (see Unit II.A.6.a "Insecticide susceptibility" in the Human Health and Environmental Risk Assessment in this docket), and such mitigation measures can be implemented.

With regard to the comment on potential for deleterious changes in genetics of wild *Ae*. *aegypti* populations, as described in the response to Unit V.A and Unit V.B, EPA concluded 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 (also see Memorandum on Vectorial Capacity located in this docket). Should any deleterious changes be observed, effective mitigation measures could be implemented as noted above.

EPA Response to Unit XII.I. – Comments that EPA Must Address Ethical Concerns: Human Subjects and Informed Consent. With regard to comments suggesting that humans present in the EUP testing area should be considered human subjects and the testing be subject to the requirements of human studies rules, EPA does not find that the research involved with Oxitec's release of male OX5034 mosquitoes meets the regulatory definition of research involving human subjects under the applicable regulatory standard, 40 CFR 26, Subparts K-L. Because the research does not include "human subjects" as defined in the regulation, the threshold of "research involving intentional exposure of human subjects" is not met, and therefore the requirements of EPA's human studies rule do not apply to this research proposed by Oxitec.

With regard to the comment (0300) referencing the Common Rule (40 CFR 26, subpart A) to support the assertion that informed consent of those living in the area of the Oxitec release must be obtained prior to initiating the research, because Oxitec is not a federal agency or

conducting research sponsored or funded by a federal agency, the Common Rule does not apply. Rather, because this a private study conducted with the intention of submitting the results to EPA in support of a pesticide registration decision, the relevant standards are found in EPA's Rule for the Protection of Human Subjects of Research (40 CFR 26, Subparts K-L). This regulation is based on the federal Common Rule and consistent with the Nuremberg Code (*see* 70 FR 53838, 53858-9; September 12, 2005). Subpart K requires that study sponsors conducting research involving intentional exposure of human subjects to any substance with the intention of submitting the results to EPA comply with protections for human subjects. These protections include obtaining informed consent of subjects, balancing risks and benefits of the research, and obtaining review of the proposed study by an independent institutional review board prior to initiating research. Subpart L prohibits conducting research subject to Subpart K if it involves pregnant or nursing women, or children.

Under 40 CFR §26.1102(l), "research involving intentional exposure of a human subject means a study of a substance in which the exposure to the substance experienced by a human subject participating in the study would not have occurred but for the human subject's participation in the study." There are three elements to this definition that all must be satisfied for the research to be subject to the requirements of 40 CFR 26, Subparts K-L:

1. Research. According to the rule, "*Research* means a systematic investigation, including research, development, testing and evaluation, designed to develop or contribute to generalizable knowledge. Activities which meet this definition constitute research for purposes of this subpart, whether or not they are considered research for other purposes. For example, some demonstration and service programs may include research activities."

2. Human subjects. "Human subject" is defined as "a living individual about whom an investigator (whether professional or student) conducting research:

   (i) Obtains information or biospecimens through intervention or interaction with the individual, or analyzes the information or biospecimens, or
   (ii) Obtains, uses, studies, analyzes, or generates identifiable private information or biospecimens."

   Further, as part of the definition of "human subject", the regulation specifies that:

   "*Intervention* includes both physical procedures by which information or biospecimens are gathered (*e.g.*, venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes.

   "*Interaction* includes communication between investigator and subject.

"*Private information* includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (e.g., a medical record).

"*Identifiable private information* is private information for which the identity of the subject is or may be readily ascertained by the investigator or associated with the information." (40 CFR 26.1102(2)-(6))

3. Intentional exposure. If it was research involving human subjects, did the research involve study of a substance in which the exposure to the substance experienced by a human subject participating in the study would not have occurred but for the human subject's participation in the study?

The release of Oxitec mosquitoes under the EUP meets the definition of research. The company is releasing the mosquitoes to gather information in a systematic manner to contribute to the generalizable knowledge on the impact of releasing genetically modified mosquitoes on the local mosquito population.

Moving to the second element of the definition of "research involving intentional exposure of human subjects," the research does not involve "human subjects" as defined by the regulation. As the focus of the release under the conditions of the EUP, Oxitec would collect information on how efficacious releases of male OX5034 mosquito are at suppressing wild *Ae. aegypti* mosquito populations in the test area. To obtain data/information about the efficacy of the release of male OX5034 mosquitoes, the company 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. Additional information on the parameters of the testing to occur under the EUP can be found in the risk assessment entitled "Review of Section G" that can be found in the docket established for this action.

During testing, releases of male mosquitoes as well as all traps and egg release boxes will be outdoors, and not inside anyone's home. In trial A, there will be a single release point and in trial B, multiple release points. With regard to the placement of the ovitraps and BG sentinel traps; all traps will be positioned outside of residences in sheltered locations, typically nearby residential, commercial, or utility premises. In trial A, the traps will be placed in a concentric circle around the single release point. In trial B, traps will be placed at the perimeter of mosquito dispersal determined in trial A. Oxitec is not proposing to collect any information about individuals in the area of the release or to monitor behavior of individuals. Oxitec also is

not proposing to gather identifiable private information about or identifiable biospecimens from anyone in conjunction with the release or to monitor the efficacy of releasing wild males. The location of houses in a specific area is not private information; it may be obtained easily through Internet searches and using publicly available satellite maps. None of the information that Oxitec proposes to gather in the course of this research involving the release of male OX5034 mosquitoes involves data about a living individual gathered through interaction with the individual, or collecting identifiable private information about or identifiable biospecimens from those who may be present in the area of the Oxitec release. Therefore, the research involved with Oxitec's release of mosquitoes does not meet the regulatory definition of research involving human subjects. Because the proposed information to be collected as part of this research does not involve human subjects, it is not necessary to evaluate whether the research would constitute intentional exposure of human subjects.

The research does not meet the definition of "research involving intentional exposure of a human subject;" therefore, it is not subject to the requirements of 40 CFR 26, Subparts K-L. This means that Oxitec is not required under EPA's human studies rule to obtain informed consent of those living in the areas where the Oxitec mosquitoes would be released under the EUP.

EPA Response to Unit XII.J. – Comments Arguing that EPA Must Prepare a Full EIS Under the National Environmental Policy Act (NEPA). Some commenters assert that although "[a]ctions under FIFRA have traditionally been exempt from NEPA, … an assessment under NEPA is … required" prior to issuance of the present EUP because "there are many issues associated with the release of GE mosquitoes into the environment which may not be adequately captured by assessment under FIFRA." (Center for Food Safety 0344 p.5-6; GeneWatch UK 0335 p. 4).

EPA disagrees with the assertion that a NEPA analysis must be conducted in this case, and with the implication that EPA is required to determine, on a case by case basis, whether certain pesticide registration actions require a NEPA analysis. To the contrary, the courts have consistently held that Congress did not intend for NEPA's requirements to apply to FIFRA's scheme for registering pesticides.

Commenters cite the case of EDF v. EPA, 489 F.2d 1247 (D.C. Cir. 1973), for the proposition that the courts have carved out a "narrow exemption" from NEPA for pesticide registration actions, suggesting that each individual pesticide registration action under FIFRA must be examined in order to determine whether the exemption applies. Commenters misconstrue the court's decision in that case and ignore subsequent judicial treatment of that case. In EDF v. EPA, the court affirmed the lower court's decision finding that an EIS was not required prior to EPA's decision to ban the use of DDT because EPA was engaged in an examination of environmental questions. The court explained:

> We are not formulating a broad exemption from NEPA for all environmental agencies or even for all environmentally protective regulatory actions of such

> agencies. Instead, we delineate a narrow exemption from the literal requirements for those actions which are undertaken pursuant to sufficient safeguards so that the purpose and policies behind NEPA will necessarily be fulfilled. The EPA action here meets this standard.

EDF v. EPA, 489 F.2d at 1257. EPA interprets this decision – as do subsequent judicial decisions applying this case – as holding that while all "environmental" decisions of U.S. government agencies are not exempt from NEPA, all pesticide registration actions under FIFRA *are* exempt from the requirements of NEPA.

EDF v. EPA was subsequently cited with approval by the court in Merrell v. Thomas, 807 F.2d 776 (9th Cir. 1986). In Merrell, the court affirmed the lower court's grant of summary judgment to EPA in a citizen's action seeking to enjoin the registration of certain herbicides, finding that Congress did not intend for NEPA to apply to pesticide registration actions taken by EPA under FIFRA. There, the court stated that "[t]he question before us is, did Congress intend to superimpose NEPA's procedures on top of the FIFRA registration procedure," *Id*. at 778, and held that "[a]fter examining FIFRA's registration procedure, its registration standard, and the applicable review procedures, we conclude that Congress did not intend that the EPA should comply with NEPA." *Id*. at 776. The court explained:

> [T]he 1972 amendments [to FIFRA] … reflected a compromise between environmentalists, farmers, and manufacturers. The differences between FIFRA's registration procedure and NEPA's requirements indicate that Congress did not intend that NEPA apply… To apply NEPA to FIFRA's registration process would sabotage the delicate machinery that Congress designed to register new pesticides. It would increase a regulatory burden that Congress intentionally lightened in 1978 and create new opportunities for litigation where litigation was recently quelled.

Id. at 778-779 (citations omitted). Citing, *inter alia*, EDF v. EPA, the Merrell court concluded:

> Our position that NEPA does not apply to pesticides registered under FIFRA has been taken by other courts as well. Speaking in terms of the "functional equivalence" of the EPA's procedures to NEPA's procedures, these courts conclude that formal compliance with NEPA would be wasteful and redundant. *E.g.,* Wyoming v. Hathaway, 525 F.2d 66, 71-72 (10th Cir. 1975) (EPA need not prepare an EIS before cancelling or suspending registrations of three coyote poisons), *cert. denied*. 426 U.S. 906, 96 S. Ct. 2226, 48 L. Ed. 2d 830 (1976); Environmental Defense Fund, Inc. v. Environmental Protection Agency, 160 U.S. App. D.C. 123, 489 F.2d 1247, 1254-56 (D.C. Cir. 1973) (EPA need not prepare an EIS before cancelling registration of DDT with respect to nearly all uses); Environmental Defense Fund, Inc. v. Blum, 458 F. Supp. 650, 661-62 (D.D.C.

> 1978) (EPA need not prepare an EIS before granting an emergency exemption to a state to use an unregistered pesticide). While we hesitate to adopt the "functional equivalence" rationale, *we are confident that Congress did not intend NEPA to apply to FIFRA registrations*.

Merrell v. Thomas, 807 F.2d at 778 (emphasis added).

Finally, the Merrell decision was more recently cited with approval by the court in San Luis & Delta-Mendota Water Authority v. Jewell, 747 F.3d 581 (9th Cir. 2014) ("[W]e do not think that the distinctions [between Section 7 of the ESA and NEPA] are as pronounced as those in *Merrell*, where the court concluded that '[t]o apply NEPA to FIFRA's registration process would sabotage the delicate machinery that Congress designed to register new pesticides.'"). San Luis & Delta-Mendota Water Authority at 650 (citation omitted).

Because Congress did not intend for NEPA requirements to apply to pesticide registration actions by EPA under FIFRA, EPA need not conduct a NEPA analysis before issuing the present EUP.

EPA is including its analysis of the OX5034 submission requesting issuance of an EUP in the docket for this action. (See EPA's response to Unit XII.A for a list of documents developed through the Agency's analysis of the EUP application requesting a permit to test OX5034.)

Comments that pertain to *Ae. aegypti* as a known disease vector are addressed in the Agency's responses to Unit VII.

EPA Response to Unit XII.K. – Comments Stating that EPA Must Comply With the Endangered Species Act. EPA has made a "no effect" finding as to threatened or endangered species under the Endangered Species Act with regard to the present EUP. See Unit II.D.3, "Impacts on endangered species," of the Human Health and Environmental Risk Assessment, which can be found in the docket established for this action.

EPA Response to Unit XII.L. – Comments Stating that EPA Must Comply with the Veterinary Feed Directive. 21 U.S.C. § 354(a)(1) states, in part, that "Any animal feed bearing or containing a veterinary feed directive drug [as defined] shall be fed to animals only by or upon a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice."

Although commenters have stated that "[i]t is … essential to consider whether Oxitec's use of antibiotics is lawful under the Veterinary Feed Directive (21 U.S.C. §354)" (GeneWatch UK 0335 p. 9), commenters have not suggested any way in which Oxitec's use of antibiotics in the production of its mosquitoes violates Veterinary Feed Directive. Further, the Veterinary Feed

Directive described at 21 U.S.C. § 354 is administered by the U.S. Food and Drug Administration (FDA), and not by the EPA. Finally, EPA notes that no antibiotics will be used in the U.S. to grow mosquitoes; only the mosquito eggs, which will not be kept in the presence of antibiotics, will be shipped to the U.S.; and antibiotics will not be used during the field trials (See Unit II.A.5. "Rearing and shipping of OX5034" and Unit II.D.2.c. "Microbes," of the Human Health and Environmental Risk Assessment. These considerations also apply to the WHO Global Action Plan for Antimicrobial Resistance.

EPA Response to Unit XII.M. – Comments Questioning Whether Oxitec Can Be Released From the Contained Use Requirements of an Import Permit. The EPA does not administer or implement Centers for Disease Control and Prevention (CDC) regulations or permits and does not purport to "release" Oxitec from any applicable requirements imposed by any CDC regulations or permits. The EUP authorizes certain actions under FIFRA. It remains Oxitec's responsibility to maintain compliance with any other applicable requirements.

EPA Response to Unit XII.N. – Comments Stating that EPA Should Not Rely on the Registrant Provided Assessment Data/Information. FIFRA and its implementing regulations evince a legislative intent and establish a regulatory scheme whereby applicants for pesticide registration actions generate the data necessary to support registration of their products. For example, FIFRA Section 3(c)(F)(1) requires applicants for registration to provide to EPA "a full description of the tests made and the results thereof upon which the claims are based, or alternatively a citation to data that appear in the public literature or that previously had been submitted to [EPA]…." Section 408(d)(2)(A) of the FFDCA similarly requires that petitions to establish a tolerance or tolerance exemption for pesticide chemical residues on food be "supported by such data and information as are specified in regulations… including … an informative summary of the … data, information, and arguments submitted or cited in support of the petition…." FIFRA section 3(c)(1)(F) goes on to provide specific protections for the rights of data submitters (i.e., applicants and registrants), which are implemented by regulations at 40 CFR Part 152, subpart E. 40 CFR 152.50(f) requires that a pesticide registration application demonstrate satisfaction of "data requirements," and 40 CFR Part 158 sets forth the "data requirements for pesticides" that applicants must satisfy (*see*, *e.g.*, 40 CFR 158.1(a) and(b), stating the "purpose" and "scope" of the Part 158 data requirements). 40 CFR 152.107 speaks to EPA "review of data" that has been "submitted or cited by an applicant." 40 CFR Part 160 "prescribes good laboratory practices for conducting studies that support or are intended to support applications for [pesticide registrations] … [and] assure the quality and integrity of data submitted pursuant to sections 3, 4, 5, 8, 18, and 24(c) of [FIFRA] and section 408 or 409 of [the FFDCA]." 40 CFR 160.1. FIFRA Section 33, setting forth "Pesticide Registration Service Fees" pursuant to the Pesticide Registration Improvement Act (PRIA), as amended, is predicated largely upon EPA review of applicant-generated and submitted studies. FIFRA Section 10 contains provisions protecting against disclosure of such "information submitted [to EPA] by an applicant or registrant." FIFRA Section 10(g)(1). This recitation of statutory and regulatory provisions evincing a legislative intent and regulatory scheme under which applicants for

pesticide registration actions generate the data necessary to support registration of their products is provided for example only and is not intended to be exhaustive.

EPA has developed rigorous methodological standards, standard evaluation procedures, and statistical review procedures to evaluate the quality and conclusions of every applicant/registrant-submitted study, and EPA has developed data quality evaluation procedures to document EPA's review and conclusions regarding data quality of all studies[15,16]. In addition, where appropriate in evaluating submissions for pesticide registration actions, EPA will also use data from sources other than the applicant/registrant, including government reports, academic submissions, and data from publicly published studies in peer reviewed scientific journals[17]. When high quality data from sources other than applicants/registrants are available and deemed appropriate for quantitative risk assessment purposes, that data has been used in place of applicant/registrant-submitted data.

Finally, Congress has not appropriated to EPA or other agencies the funding to do the studies and generate the data necessary to support pesticide registrations, and the financial cost of the data is significant. Rather, as described above, FIFRA and its implementing regulations evince a legislative intent and establish a regulatory scheme whereby applicants for pesticide registration actions generate the data necessary to support registration of their products, and EPA rigorously evaluates such data.

[15] 40 CFR Part 158.2080 - Experimental use permit data requirements – biochemical pesticides.
[16] Memorandum: R. McNally to M. Mendelsohn and S. Borges. BPPD Guidance for Senior Staff and Branch Chief Review of Guidance Documents. August 14, 2018.
[17] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-identifying-selecting-and-evaluating-open (last accessed: 04.22.2020)

APPENDIX

Number	Commenter	Organization
0003	Anonymous	
0004	Anonymous	
0005	Anonymous	
0006	K.S. Wasserman	
0007	Anonymous	
0008	Anonymous	
0009	Anonymous	
0010	P. Kircher	
0011	A. Conover	
0012	Anonymous	
0013	Anonymous	
0014	Anonymous	
0015	Anonymous	
0016	Anonymous	
0017	Anonymous	
0018	Q. Perkins	
0019	Anonymous	
0020	Anonymous	
0021	Anonymous	
0022	Anonymous	
0023	Anonymous	
0024	T. Nolan	
0025	Anonymous	
0026	Anonymous	
0027	S.C. Ray	
0028	Anonymous	
0029	Anonymous	
0030	E. Young	
0031	Anonymous	
0032	Anonymous	
0033	L. R. Marshall	
0034	Anonymous	
0035	L. Jones	
0036	Anonymous	
0037	J. W. Norris	
0038	B. Wray	
0039	Anonymous	
0040	Anonymous	
0041	Anonymous	

Number	Commenter	Organization
0042	Anonymous	
0043	Anonymous	
0044	Anonymous	
0045	Anonymous	
0046	Anonymous	
0047	Anonymous	
0048	Anonymous	
0049	A. Tweedale	
0050	Anonymous	
0051	Anonymous	
0052	H. Scott	
0053	Anonymous	
0054	K. Grens	
0055	Mindful Dissenters	
0056	Anonymous	
0057	R. Knight	
0058	K. Pierson	
0059	Anonymous	
0060	Moms Across America	
0061	Anonymous	
0062	J. Thebeau	
0063	J. Courtney	
0064	M. Corbett	
0065	Anonymous	
0066	C. Harman	
0067	Anonymous	
0068	P.L. Goodman	Florida Keys Mosquito Control District
0069	Anonymous	
0070	Anonymous	
0071	B. VanGheluwe	BVG Law Offices
0072	Anonymous	
0073	Anonymous	
0074	Anonymous	
0075	Anonymous	
0076	Anonymous	
0077	Anonymous	
0078	D. Fyke	
0079	Anonymous	
0080	Anonymous	
0081	R. Stephens	
0082	Anonymous	

Number	Commenter	Organization
0083	Anonymous	
0084	Anonymous	
0085	Anonymous	
0086	Anonymous	
0087	Anonymous	
0088	Anonymous	
0089	J. Butler	
0090	J. Conrow	
0091	Anonymous	
0092	G. Morales	
0093	A. Glenn	
0094	Anonymous	
0095	J. Smith	
0096	Anonymous	
0097	J. Birk	
0098	Anonymous	
0099	S.L. Smith	
0100	Anonymous	
0101	Anonymous	
0102	S. Black	
0103	Anonymous	
0104	Anonymous	
0105	C.M. Burke	
0106	C. Tong	
0107	C. Tong	
0108	K. Minter	
0109	Eileen	
0110	Anonymous	
0111	Anonymous	
0112	Anonymous	
0113	Anonymous	
0114	A. Hart	
0115	Anonymous	
0116	Anonymous	
0117	Anonymous	
0118	Anonymous	
0119	Anonymous	
0120	Anonymous	
0121	Anonymous	
0122	K. Folta	
0123	Anonymous	

Number	Commenter	Organization
0124	Anonymous	
0125	Anonymous	
0126	K. Bell	
0127	Anonymous	
0128	Anonymous	
0129	L. Wilcox	
0130	Anonymous	
0131	Anonymous	
0132	A. Johnson	
0133	Anonymous	
0134	Anonymous	
0135	J. Butler	
0136	Anonymous	
0137	Anonymous	
0138	Harvig Family	
0139	Anonymous	
0140	K. Grens	
0141	Anonymous	
0142	R. Smith	
0143	Anonymous	
0144	T. Wright	
0145	Anonymous	
0146	Anonymous	
0147	Anonymous	
0148	Anonymous	
0149	Anonymous	
0150	S. Appemane	M/s GBIT
0151	Anonymous	
0152	Anonymous	
0153	Anonymous	
0154	K. Later	
0155	Anonymous	
0156	Anonymous	
0157	Anonymous	
0158	Anonymous	
0159	Anonymous	
0160	Anonymous	
0161	Anonymous	
0162	Anonymous	
0163	Anonymous	
0164	Anonymous	

Number	Commenter	Organization
0165	D. Scott	
0166	Anonymous	
0167	Anonymous	
0168	Anonymous	
0169	Anonymous	
0170	M. Blaney	
0171	Anonymous	
0172	Anonymous	
0173	Anonymous	
0174	D.S. Wilde	
0175	Anonymous	
0176	Anonymous	
0177	Anonymous	
0178	Anonymous	
0179	Anonymous	
0180	Anonymous	
0181	Anonymous	
0182	Anonymous	
0183	D. Miller	
0184	Anonymous	
0185	Anonymous	
0186	Anonymous	
0187	Anonymous	
0188	Paul B.	
0189	Anonymous	
0190	Anonymous	
0191	M. Coldiron	
0192	Anonymous	
0193	G.E. DelVecchio	
0194	Anonymous	
0195	Anonymous	
0196	M. LaVeau	
0197	Anonymous	
0198	Anonymous	
0199	Anonymous	
0200	H.M.	
0201	Anonymous	
0202	Anonymous	
0203	Anonymous	
0204	Anonymous	
0205	Anonymous	

Number	Commenter	Organization
0206	Anonymous	
0207	A. Shelton	
0208	Anonymous	
0209	Anonymous	
0210	D. Bell	
0211	Anonymous	
0212	Anonymous	
0213	Anonymous	
0214	Anonymous	
0215	Anonymous	
0216	Anonymous	
0217	Anonymous	
0218	E. Davidson	
0219	Anonymous	
0220	Anonymous	
0221	Anonymous	
0222	Anonymous	
0223	T. Ritchie	
0224	Anonymous	
0225	Anonymous	
0226	Anonymous	Oxitec's Failed GM Mosquito Releases Worldwide
0227	Anonymous	
0228	Anonymous	
0229	A. Parker	
0230	Anonymous	
0231	Anonymous	
0232	A. Vaughn	
0233	Anonymous	
0234	Anonymous	
0235	R. Marquardt III	
0236	Anonymous	
0237	Anonymous	
0238	D. Mader	
0239	Anonymous	
0240	Anonymous	
0241	Anonymous	
0242	Anonymous	
0243	Anonymous	
0244	Anonymous	
0245	Anonymous	
0246	Anonymous	

Number	Commenter	Organization
0247	Anonymous	
0248	Anonymous	
0249	Anonymous	
0250	Anonymous	
0251	Anonymous	
0252	Anonymous	
0253	C. Overly	
0254	D. Cowden	
0255	Anonymous	
0256	M. Rozier	
0257	D. Fellows	
0258	H. Sapp	
0259	Anonymous	
0260	Anonymous	
0261	Anonymous	
0262	Anonymous	
0263	J.M. Conlon	American Mosquito Control Association
0264	J. Berman Diaz	
0265	Anonymous	
0266	C. Cheromiah	
0267	Anonymous	
0268	N. Adams	
0269	V. Hart	
0270	Smith	
0271	J.A. Singleton	
0272	Anonymous	
0273	G. Lee	
0274	T. Kelley	
0275	Anonymous	
0276	B. Daughtry	
0277	Sandhill Organics	
0278	Anonymous	
0279	Claudia	
0280	Anonymous	
0281	Anonymous	
0282	Anonymous	
0283	Anonymous	
0284	Anonymous	
0285	Anonymous	
0286	Anonymous	
0287	Anonymous	

Number	Commenter	Organization
0288	Anonymous	
0289	Anonymous	Dead link (Wrong docket?)
0290	J.W. Norris III	
0291	T. Tokuda	Comment on mining in Australia (Wrong docket)
0292	Anonymous	
0293	J.W. Norris III	
0294	J.W. Norris III	
0295	J. Barton	
0296	Anonymous	
0297	Anonymous	
0298	Anonymous	
0299	Anonymous	
0300	M. Daly	
0301	D. Strickman	
0302	Anonymous	
0303	Anonymous	
0304	Anonymous	
0305	P. Larry	
0306	M. Hull	
0307	Deef	
0308	A. Purkis	
0309	M Thomas	Toxic Systems Information Agency
0310	Anonymous	
0311	Anonymous	
0312	Anonymous	
0313	Anonymous	
0314	Anonymous	
0315	Hasham	
0316	C. Lish	
0317	D. Rubin	
0318	J. Rubin	
0319	Anonymous	
0320	K. Gould	
0321	Anonymous	
0322	Anonymous	
0323	Anonymous	
0324	N.C. Leppla	U of FL Integrated Pest Management Program
0325	J.M. Conlon	American Mosquito Control Association
0326	GMO Free USA	
0327	W. Jordan & A. Jones	
0328	Anonymous	

Number	Commenter	Organization
0329	Anonymous	
0330	Anonymous	
0331	B. Wray	Florida Keys Environmental Coalition
0332	L. M. Castro	
0333	L. Sanders	
0334	J.W. Norris III	
0335	H. Wallace	GeneWatch UK
0336	C. Nesbitt	BIO
0337	R.E. Goodman	University of Nebraska
0338	J.M. Conlon	American Mosquito Control Association
0339	Anonymous	
0340	Aline DeLucia	NASDA
0341	N. Rose	Oxitec Ltd
0342	Dana Perls	Friends of the Earth
0343	J. Morris	International Center for Law & Economics
0344	J. Hanson	Center for Food Safety
0345	Dana Perls	Friends of the Earth
0346	Anonymous	
0347	Anonymous	
0348	Anonymous	
0349	Anonymous	
0350	Anonymous	