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I. Introduction

In a Federal Register Notice published on August 31, 2021 (86 FR 48691), the Environmental Protection Agency (EPA or the Agency) announced the receipt of an application 93167-EUP-2 from Oxitec, Ltd., (Oxitec) requesting an amendment and extension to the experimental use permit (EUP), issued on April 30, 2020, for the OX5034 *Ae. aegypti* mosquitoes expressing tetracycline Trans-Activator Variant (tTAV-OX5034) protein as a tool for suppression of wild *Ae. aegypti* mosquito populations. The Agency has determined that the permit may be of regional and national significance; therefore, EPA invited comments on this application to amend and extend the EUP.

Oxitec Ltd. originally proposed to extend testing of OX5034 *Ae. aegypti* mosquitoes expressing the tTAV-OX5034 protein for 2 years in the state of Florida on up to 6,240 total acres at a maximum rate of 0.000082 g active ingredient (tTAV-OX5034), equivalent to 20,000 male OX5034 mosquitoes, per acre, per week. Additionally, Oxitec Ltd. had proposed to expand testing of OX5034 *Ae. aegypti* mosquitoes expressing tTAV-OX5034 protein in the state of California on up to 84,600 total acres at a maximum rate of 0.000123 g active ingredient, equivalent to 30,000 male OX5034 mosquitoes, per acre, per week. Oxitec subsequently modified this request to reduce the proposed acreage to 5,360 total acres in Monroe County, Florida and 29,400 total acres in Stanislaus, Fresno, Tulare and San Bernardino counties in California. The proposed experiments are to evaluate the efficacy of OX5034 mosquitoes as a tool for suppression of wild *Ae. aegypti* mosquito populations. EPA made its decision to grant the original Oxitec OX5034 Mosquito Experimental Use Permit on April 30, 2020 after extensive evaluation of the best available science, and after seeking and addressing public input.

II. Overview of Comments Received on the Notice of Receipt

This document summarizes comments that EPA received in response to the August 31, 2021 Federal Register Notice (86 FR 48691) announcing EPA's receipt of an application 93167-EUP-2 from Oxitec, Ltd. requesting an extension and amendment to the experimental use permit (EUP) for the OX5034 *Ae. aegypti* mosquitoes expressing tetracycline Trans-Activator Variant (tTAV-OX5034) protein.
Twelve thousand, nine hundred and sixty-one (12,961) individuals responded to the Federal Register Notice. EPA thanks all commenters for their participation in the public process.

Comments were received from industry, academia, professional and trade associations, state regulatory authorities, public interest groups and private citizens. One hundred comments were posted for public view at https://www.regulations.gov/. Some of these comments were from single respondents, while some entries recorded in the public-view docket contained multiple signatures. Four of the posted comments carried hundreds of signatures: Friends of the Earth (0462) submitted a comment with 4,258 signatures; the Institute for Responsible Technology (0461) submitted a comment with 3,336 signatures; the Center for Food Safety (0463) submitted one comment with 313 signatures and a second with 1,106 additional signatures (0464). In addition, another group of 3,852 identical comments were received as

part of a mass mail campaign. As these 3,852 comments were identical, only one of these comments (0460) was made available in public view at https://www.regulations.gov/. In this Response to Comments document, EPA treats entry 0460 as representing all of the 3,852 identical comments received. An index relating the four-digit number associated with an entry to the entities making the entry into the docket can be found in the Appendix to this document.

In addition, the Center for Food Safety (0396) requested that comments that the Center had previously submitted on October 11, 2019 (0344) in response to the Notice of Receipt for the 2019 application for an EUP to evaluate the efficacy of OX5034 mosquitoes (identifier 93167-EUP-E) be incorporated by reference into the record for the current Notice of Receipt for application 93167-EUP-2 stating that:

> "We hereby incorporate by reference our October 11, 2019, comments on the original Experimental Use Permit (EUP) for the release of Oxitec OX5034 GE mosquitoes, under this same Docket number. Our concerns raised in those comments remain as none of them have been properly addressed and are applicable to this proposed amendment and extension of the EUP." (0396 p. 1)

In light of the Center for Food Safety request, EPA considers those previous comments to be incorporated into the record for the current Notice of Receipt for application 93167-EUP-2. However, the Center for Food Safety does not specify in what way it believes those comments to have been inadequately addressed, and EPA believes that its previous response to those comments (EPA-HQ-OPP-2019-0274-0355) is complete and accurate.

Comments quoted in this Response to Comment document were chosen to illustrate points made in comments relevant to issues related to determining whether to permit additional testing of OX5034 by amending and extending the existing EUP. Some comments that were received simply express an opinion without providing sufficient information to illustrate points associated with issues relevant to determining whether to permit additional testing of OX5034 or to allow the Agency to formulate a response. Comments in this category, while included in the index, may not be quoted in this document. All comments received have been considered and the entry identifier tabulated in the index attached as an Appendix to this document.

A. Comments Supporting Amendment and Extension of the EUP for Testing of OX5034

In toto, 34 commenters expressed support for the amendment and extension of the EUP for testing of OX5034. Twenty-five entries supporting amendment and extension of the EUP and testing of OX5034 were recorded in the public-view docket. (0365-1, 0365-2, 0366, 0367, 0374, 0375, 0377, 0381, 0385, 0386, 0389, 0390, 0391, 0392, 0393, 0395, 0398, 0403, 0415, 0421, 0422, 0427, 0429, 0437, 0459). Comments were chosen from among the comments expressing support to represent the full range of these comments. Responses to comments offered in these entries can be found in Unit III of this Response to Comment document.

B. Comments Expressing Opposition to Amending and Extending the EUP

In toto, 12,927 commenters expressed opposition. Seventy-six entries were posted in the public-view docket urging the EPA to reject the request to amend and extend the EUP (0368, 0369, 0370, 0371, 0372, 0373, 0376, 0378, 0379, 0380, 0382, 0383, 0384, 0387, 0388, 0394, 0396, 0397, 0399, 0400, 0401, 0402, 0404, 0405, 0406, 0407, 0408, 0409, 0410, 0411, 0412, 0413, 0414, 0416, 0417, 0418, 0419, 0420, 0423, 0424, 0425, 0426, 0428, 0430, 0431, 0432, 0433, 0434, 0435, 0436, 0438, 0439, 0440, 0441, 0442, 0443, 0444, 0445, 0446, 0447, 0448, 0449, 0450, 0451, 0452, 0453, 0454, 0455, 0456, 0457, 0458, 0460, 0461, 0462, 0463, 0464). Included in the total of 12,927 commenters expressing opposition are the 3,852 entries that were identical to entry 0460 but that were not made available in public view at https://www.regulations.gov/, as well as the entry with 4,258 signatures submitted by Friends of the Earth (0462); the entry with 3,336 signatures submitted by the Institute for Responsible Technology (0461); and the entries with 313 signatures and 1,106 additional signatures submitted by the Center for Food Safety (0463 & 0464). Comments offered in opposition covered a range of topics.

Some of the comments received in opposition were generic in nature, offering no substantive explanation for opposition. Others, however, provided technical, legal or other arguments for opposing extending and amending the 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 amend and extend the permit for OX5034 testing. Some commenters offered opinions on what the Agency needs to do to ensure safety and the validity of the testing under the EUP.

Comments were chosen from among the comments expressing opposition to represent the full range of issues raised in all of the comments. This includes the request from the Center for Food Safety (0396) that comments the Center had previously submitted in response to the Notice of Receipt for the 2019 EUP (0344) be incorporated by reference into the record for the current Notice of Receipt. Responses to comments submitted by the Center for Food Safety on October 11, 2019 (0344) were initially addressed in the EPA's 2020 Response to Comment document (0355).

EPA responses to the full range of issues can be found in Units IV through X of this Response to Comments document.

III. Comments Supporting Amending and Extending the EUP

Comments supporting (0365-1, 0365-2, 0367, 0374, 0375, 0377, 0381, 0385, 0386, 0389, 0390, 0391, 0392, 0393, 0398, 0403, 0407, 0422, 0429, 0437, 0459) extending and amending the EUP issued in April 2020 to permit testing of OX5034 mosquito generally revolve around: (1) the need to control *Ae. aegypti* including a description of the diseases vectored by *Ae. aegypti*; (2) the characteristics of *Ae. aegypti* mosquito affecting the efficacy of measures used to control the mosquito; (3) *Ae. aegypti* mosquito spread; (4) limitations of existing control methods; and (5) potential benefits of OX5034.

A. Comments Describing Why Control of *Ae. aegypti* is Needed

Comments in this category (0365-1, 0365-2, 0367, 0377, 0386, 0392, 0398, 0459) revolve around the invasive nature of *Ae. aegypti*, the rapid spread of *Ae. aegypti* in the U.S. and the status of the *Ae. aegypti* mosquito in California and the southeastern US. Also included are comments (0365-2, 0386, 0398, 0459) on the potential for climate change to expand the range of *Ae. aegypti*.

1. *Ae. aegypti* is an invasive species posing a threat to humans and other animals

Several comments (0377, 0386, 0459) explained the risks that *Ae. aegypti* poses to humans and other animals.

Michael Weissmann, Ph. D. Entomology, Kallima Consultants, stated that:

> "Aedes aegypti is an invasive, introduced mosquito species that does not belong in the United States. It is a competent vector of many human diseases including Yellow Fever, Dengue, Chikungunya, Zika virus, and others. Unfortunately, this dangerous species has become established in many locations in the southern United States." (0459 p. 1)

Julian Morris, the Reason Foundation, described *Ae. aegypti* as:

> *"*. . . is responsible for numerous serious—and often fatal—viral diseases, including yellow fever . . .dengue . . . chikungunya . . . Zika. . . . While the incidence of mosquito-borne disease in the U.S. is a tiny fraction of what it once was, it remains a threat both in the continental U.S. and in U.S. territories. . . . There have been numerous outbreaks of diseases spread by *Aedes aegypti* in the continental U.S. and in U.S. territories over the past two decades." (0377 pp. 2-3) [Footnote Omitted]

Mark van der List, a veterinarian in California, seeing "firsthand the need to protect animals from debilitating and deadly mosquito transmitted diseases," stated that:

> "Invasive mosquitoes are becoming an increasing threat in California from both a veterinary and human health perspective. As a veterinarian, I am concerned about heartworm disease in dogs, cats, and ferrets which can be transmitted by *Aedes aegypti* mosquitoes. Heartworm is a very serious and potentially fatal disease and I support efforts to reduce the threat posed by invasive *Aedes aegypti* mosquitoes . . . The threat of heartworm disease in pets is pervasive in California. . . . , watering practices in western desert communities have created conditions conducive to mosquito proliferation." (0386 p. 1)

Julian Morris, the Reason Foundation, described *Ae. aegypti* as:

> *".* . . one of the most widespread mosquito species globally . . ." (0377 p. 2-3)

2. Rapid spread of *Ae. aegypti* in the U.S.

Four comments (0365-1, 0367, 0377, 0392) addressed the speed with which *Ae. aegypti* mosquito is spreading in the US, including the rapid increase of the mosquito's range in California and the re-emergence populations of the *Ae. aegypti* mosquitoes in the southeastern US.

The Mosquito and Vector Control Association of California (MVCAC), comprised of approximately 70 California mosquito control and public health agencies, stated that:

> "Invasive *Aedes aegypti* mosquitoes were detected in California in 2013 and are now in over 20 counties throughout the state ranging from Shasta County in the north to San Diego and Imperial Counties near the Mexican border." (0365-1 p. 1)

Mustapha Debboun, General Manager of Delta Mosquito & Vector Control District in Visalia California, also stated that:

> "Invasive Ae. aegypti mosquitoes are now in over 20 counties throughout California" (0367 p .1)

Dan Killingsworth, Director of Operations for Environmental Security Pest Control, stated that:

> "The recent reintroduction of *Aedes aegypti* on the Florida panhandle and southern Alabama, after over twenty years of non-detection through surveillance, is an indication of the continual threat this species presents to all regions of the country." (0392 p. 1)

Julian Morris, the Reason Foundation, stated that:

> "The U.S. Centers for Disease Control (CDC) estimates that *Aedes aegypti's* range extends throughout most of the Southern and eastern U.S." (0377 p. 3)

Julian Morris, the Reason Foundation, added that:

> "The California Department of Public Health recently produced a map showing that *Aedes aegypti* mosquitoes are present in over 20 counties in California, from Shasta in the North to San Diego and Imperial in the South, . . ." (0377 p. 4)

Julian Morris, the Reason Foundation, commented that:

> "In the past 20 years, there have been several outbreaks of locally transmitted dengue in Baja California Sur, Mexico, the most recent being in 2020. Given the prevalence of Aedes aegypti (and to a lesser extent Aedes albopictus) in California, there is a considerable risk that the disease will spread north and become endemic in the US. Likewise, chikungunya appears to have gained a foothold in Baja California Sur and there is a risk that it, too, could spread north." (0377 p. 4) [Footnote Omitted]

3. Potential effects of climate change on *Ae. aegypti* spread

Some comments (0365-2, 0386, 0398, 0459) predicted that the changing climate would facilitate the spread of *Ae. aegypti* into new areas.

District and General Managers of several California mosquito and vector control agencies (0365-2), voicing support for amending and extending the EUP, stated that:

> "With a changing climate and increasing global travel and trade, there has been a worldwide expansion of invasive mosquito species and the diseases they carry. This is evidenced by the rapid spread of *Aedes aegypti* mosquitoes in the western U.S. *Aedes aegypti* are now found in over 300 cities throughout California and the CDC projects that *Aedes aegypti* will continue to expand its range into new areas across the U.S." (0365-2 p. 1)

The National Association of County and City Health Officials (NACCHO) stated that:

> "Climate change also has the potential to expand mosquito habitats and possibly introduce the risk of serious mosquito-borne diseases into areas that have not had to respond to them before." (0398 p. 1)

Mark van der List stated that:

> " . . . our state continues to see the effects of climate change, which is exacerbating the spread of invasive mosquitoes..." (0386 p. 1)

Michael Weissmann, Ph.D. Entomology, Kallima Consultants, stated that:

> "Since these mosquitoes are closely associated with human habitation, their population increases with expanded urban and suburban development." (0459 p. 1)

B. Comments Describing Incidence of Diseases Vectored by *Ae. aegypti*

Comments in this category (0374, 0377, 0398, 0422) reported on incidence of diseases caused by viruses vectored by *Ae. aegypti* mosquito.

The National Association of County and City Health Officials, an organization that works with and advocates for nearly 3,000 local health departments across the US, arguing that "[M]osquito-borne illness presents a risk to many of their communities," stated that:

> "The Centers for Disease Control and Prevention has recorded routine incidence of mosquito-borne diseases such as West Nile Virus, dengue fever, and chikungunya. The female *Aedes aegypti* mosquito represents the main vector for transmission of dengue fever and chikungunya diseases, as well as the Zika epidemic in 2016-17, and as such interventions that can reduce the female *Ae. aegypti* population can help protect public health." (0398 p. 1)

Julian Morris, the Reason Foundation, stated that:

> "There have been numerous outbreaks of diseases spread by *Aedes aegypti* in the continental U.S. and in U.S. territories over the past two decades, including:
>
> • Seasonal outbreaks of dengue in the U.S. territories of Puerto Rico, the U.S. Virgin Islands, Samoa and Guam. There was also a dengue outbreak in south Texas in 2005. And there have been frequent cases of local-transmission (albeit in relatively small numbers) in Florida.
>
> • Numerous outbreaks of chikungunya. From 2006 to 2013, an average of 28 people per year in the U.S. tested positive for chikungunya, including locally transmitted cases in Florida, Puerto Rico, and the U.S. Virgin Islands. In 2014, there was a major outbreak in U.S. Territories, leading to at least 4,659 locally-transmitted cases, as well as 12 locally-transmitted cases in Florida. The number of locally-transmitted cases has been falling since then, with 237 reported in 2015, 181 in 2016, 39 in 2017, 8 in 2018, 2 in 2019, and none in 2020 or so-far in 2021. Nonetheless, the potential for future outbreaks remains.
>
> • Sporadic outbreaks of Zika, including in Hawaii, Florida, and Texas, as well as several U.S. territories. In 2017, 433 cases of Zika virus disease cases were reported in the 50 states, including 5 cases acquired through local mosquito-borne transmission in Florida and Texas. In the same year, almost 654 locally-acquired infections occurred in U.S. territories. Since then there have been no reported instances of Zika resulting from local mosquito-borne transmission in the continental U.S. However, there have been nearly 300 cases of locally transmitted Zika in U.S. territories, most of them in Puerto Rico." (0377 p. 4) [Footnotes Omitted]

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD), stated that:

> "FKMCD is consistently rated one of the top mosquito control districts in the USA. Regardless of our qualifications, we have had outbreaks of dengue fever in 2009, 2010 and 2020 in the Florida Keys. In the southernmost part of South Florida, which consists of the Florida Keys and Miami Dade County, we have increasing numbers of the *Aedes aegypti* mosquito and have had

> outbreaks of either dengue fever or Zika in seven of the past twelve years. This has been exacerbated weekly by hundreds of flights and thousands of visitors coming here from countries where these diseases are endemic. Public health concerns in South Florida are not just concerns, they are realities we live with and seek solutions for every day." (0374 p. 1)

Andrea Leal, Executive Director of the Florida Keys Mosquito Control District, stated that:

> "As recently as 2020, we have seen just under 70 cases of locally-acquired Dengue Fever in our jurisdiction." (0422 p. 1)

C. Comments Describing the Limitations of Existing Control Methods

Comments in this category (0365-1, 0365-2, 0366, 0374, 0375, 0377, 0381, 0385, 0389, 0393, 0398, 0459) describe why new tools are needed to control *Ae. aegypti* mosquitoes and the difficulties involved in controlling *Ae. aegypti* mosquitoes with currently available technologies. Comment was also received on the emergence of resistance to the conventional pesticides commonly used in control efforts and the behavior of the mosquito itself.

1. <u>Need for better methods of control</u>

Four comments (0374, 0377, 0385) addressed the reasons behind a need for better methods of control of *Ae. aegypti*.

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD), also explained that:

> "Most of the diseases this mosquito vectors have no vaccines or therapeutic treatments available so the only way to control the viruses they carry is to control the mosquito." (0374 p. 1)

Julian Morris, the Reason Foundation, stated that:

> "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." (0377 p. 4)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD) and the Florida Mosquito Control Association and the Florida Mosquito Control Foundation, explained:

> " . . . the *Aedes aegypti* mosquito, which is a growing public health threat in the Florida Keys as well as in many parts of the US and the world, is not adequately controlled by conventional mosquito control methods." (0374 p .1)

The Florida Mosquito Control Association and the Florida Mosquito Control Foundation stated that:

> "The *Aedes aegypti* mosquito is a chronically difficult mosquito species to control with traditional techniques (ie. Source reduction, larviciding and adulticiding activities) for a number of various biological and human-societal reasons." (0385 p. 1)

2. Difficulties *Ae. aegypti* behavior presents to control

Five comments (0365-1, 0365-2, 0375, 0393, 0459) described how the behavior of *Ae. aegypti* affects control efforts.

Norman Leppla, Integrated Pest Management University of Florida, stated that:

> "The efficacy of traditional mosquito methods to control *Aedes aegypti* is often limited as these mosquitoes breed close to people's homes where it is difficult to inspect and treat." (0375 p. 1)

The Mosquito and Vector Control Association of California (MVCAC) stated that:

> "Even identifying their mosquito habitat can be difficult because they lay their eggs in small, cryptic sources. Due to the difficult nature of controlling this species and the public health risk it poses, mosquito agencies need new control methods." (0365-1 p. 1)

District and General Managers of several California mosquito and vector control agencies (0365-2), voicing support for amending and extending the EUP, stated that:

> "This mosquito exploits small water sources commonly found around the home and prefers to feed on people. Therefore, it poses considerable human health risks with the potential for local transmission of exotic viruses that cause yellow fever, Zika, dengue, and chikungunya." (0365-2 p. 1)

Referring to *Ae. aegypti*, Michael Weissmann, Ph. D. Entomology, Kallima Consultants, stated that:

> "Their larvae inhabit small containers of water and are therefore challenging to control using standard mosquito larval control methods that are focused on applying larvicides to larger bodies of water. The adults are day-flyers, such that typical adulticide applications that occur in the evenings are not effective at controlling Ae. aegypti adults." (0459 p. 1)

Several entities involved in mosquito control efforts, American Mosquito Control Association, Anastacia Mosquito Control District, Delta Mosquito and Vector Control District, Entomological Society of America, Mosquito and Vector Control Association of California, Northwest Mosquito and Vector Control Association, Society of Vector Ecology, stated that:

> "These mosquitoes and [sic] often lay their eggs in small, cryptic water sources in residential areas which makes inspection and treatment very difficult." (0393 p. 1)

3. Limitations on use of current methods of controlling *Ae. aegypti* including emergence of resistance

Ten comments (0365-1, 0365-2, 0374, 0375, 0377, 0381, 0389, 0393, 0398, 0459) described the limitations on the effectiveness of current control measures.

Referring to *Ae. aegypti*, Michael Weissmann, Ph. D. Entomology, Kallima Consultants, stated that because the "adults are day-flyers":

> ". . . typical adulticide applications that occur in the evenings are not effective at controlling Ae. aegypti adults. However, pesticide fogging in the daytime to target Ae. aegypti would negatively impact many other insects, including butterflies and day-flying pollinators such as bees. To summarize, these non-native mosquitoes are a challenge to control using standard mosquito abatement methods." (0459 p. 1)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD), explained that:

> "Adequate control is further complicated by the difficult to treat habitats preferred by the *Aedes aegypti* mosquito and the fact that the best pesticides used for mosquitoes are no longer very effective against the *Aedes aegypti* mosquito due to its increasing resistance to them. It is very clear we need new technology at mosquito control and there are not many options available to us." (0374 p .1)

Several entities involved in mosquito control efforts, American Mosquito Control Association, Anastacia Mosquito Control District, Delta Mosquito and Vector Control District, Entomological Society of America, Mosquito and Vector Control Association of California, Northwest Mosquito and Vector Control Association, Society of Vector Ecology, stated that *Ae. aegypti*:

> " . . . are becoming increasingly resistant to commonly used insecticides." (0393 p. 1)

The Mosquito and Vector Control Association of California (MVCAC), comprised of approximately 70 California mosquito control and public health agencies, stated that:

> "One of the main challenges with controlling invasive *Aedes* aegypti is that they are becoming increasingly resistant to available public health pesticides." (0365-1 p. 1)

Norman Leppla, Integrated Pest Management University of Florida, stated that:

> ". . . , this species has become resistant to some pyrethroids, the most commonly used class of mosquito adulticide." (0375 p. 1)

Julian Morris, the Reason Foundation, stated that:

> "Moreover, the repeated use of chemical insecticides is leading to rising resistance worldwide, creating challenges for mosquito control programs." (0377 pp. 4-5) [Footnote Omitted]

The American Mosquito Control Association (AMCA) stated that:

> "*Aedes aegypti* are spreading rapidly in California and their difficult nature to control and increasing resistance to pesticides necessitates innovative mosquito control interventions. . . . Mosquito control agencies throughout the United States are in desperate need of new tools to address invasive mosquito populations. Pesticide resistance has placed many agencies responsible for protecting the public health from mosquitoes, and the pathogens they may carry, in a precarious situation. As a result, the dwindling options of control alternatives need to be augmented in order for control of vector-borne diseases to be realized." (0381 & 0389 p. 1)

District and General Managers of several California mosquito and vector control agencies (0365-2), voicing support for amending and extending the EUP, stated that:

> "Traditional mosquito control strategies show variable and often limited effectiveness against *Aedes aegypti.* According to the World Health Organization's guidance framework for testing genetically modified mosquitoes, *Aedes aegypti* have demonstrated resistance to many commonly used insecticides in the Americas, Asia, and Africa. The public health need to control this invasive mosquito urges us to consider new control methods." (0365-2 p. 1)

The National Association of County and City Health Officials (NACCHO), arguing that "[M]osquito-borne illness presents a risk to many of their communities," stated that:

> "NACCHO supports the evaluation of safe and well-regulated novel vector control technologies that have the potential to protect public health and fill the gaps in current vector control strategies, such as technologies that can address the issue of insecticide resistance, which limits the effectiveness of current chemical control strategies." (0398 p. 1)

4. Economics of *Ae. aegypti* control

Three comments (0366, 0374, 0377) described the current monetary costs associated with attempting to control *Ae. aegypti* mosquitos.

Julian Morris, the Reason Foundation, 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 in the *Aedes aegypti* population." (0377 pp. 4-5) [Footnote Omitted]

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD), stated that:

> "Also, over the past ten years, FKMCD and our chemical suppliers have developed more effective and more costly chemical treatments for the *Aedes aegypti* mosquito than those used for nuisance mosquitoes. Our nuisance mosquitoes are more easily controlled by conventional pesticides including larvicides. These new chemical systems have allowed us to improve our control of the *Aedes aegypti* population to at best 50%. This level, which is the highest we have ever achieved, is still far from adequate to control local disease transmission. Our pesticide spraying for the *Aedes aegypti* mosquito has increased from ten percent of our total pesticide use on a cost basis in 2011 to more than fifty percent on a cost basis in 2020. Similar increases are also reflected in our budgets for 2021 and 2022. In years where there is local disease transmission, the amount of chemical spraying to control this menace could be much higher. These increases are significant but even more so when you consider that the *Aedes aegypti* mosquito constitutes only between two to four percent of our total mosquito population in the Keys." (0374 p. 2)

Anonymous (0366) stated that:

> "The ankle biters are getting worse and worse every year and no amount of spraying regular pesticides seem to help." (0366 p. 1)

D. Potential Benefits of OX5034

Comments (0365-1, 0365-2, 0367, 0375, 0377, 0381, 0385, 0386, 0389, 0390, 0391, 0393, 0403, 0421, 0422, 0437, 0459) in this category revolved around (1) how OX5034 might be another tool in the effort

to control *Ae. aegypti*; (2) the role OX5034 could play in Integrated Vector Management; and (3) potential benefits of OX5034 to the environment.

1. OX5034 might be another tool in the effort to control *Ae. aegypti*

Seven comments (0375, 0381, 0385, 0389, 0390, 0391, 0422) voiced the opinion that OX5034 could be another tool in the effort to control *Ae. aegypti*.

Norman Leppla, Integrated Pest Management University of Florida, stated that:

> "Oxitec's technology has the potential to provide California's communities with an innovative, effective, and environmentally sustainable *Aedes aegypti* control solution at a time when other vector management tools face challenges. . . . Approval of Oxitec's EUP amendment will enable local mosquito control districts to assess the efficacy and potential of Oxitec's technology to control this public health vector." (0375 p. 1)

The Florida Mosquito Control Association and the Florida Mosquito Control Foundation stated that:

> "The *Aedes aegypti* mosquito is a chronically difficult mosquito species to control with traditional techniques. . . . The development, research and implementation of novel control techniques is imperative to advance the scientific parameters of the public-health mosquito control industry. Limiting mosquito populations through SIT is a valuable tool for control of not only Dengue but also Zika, Chikungunya and Yellow Fever." (0385 p. 1)

The American Mosquito Control Association (AMCA) stated that:

> "This technology will not only provide another tool to be used to protect public health but will offer a potentially viable alternative to the use of pesticides to reduce adult mosquito populations in the future." (0381 & 0389 p. 1)

The Biotechnology Innovation Organization (BIO) stated that:

> "BIO strongly supports the development of innovative products to address issues of critical importance to society, including those like the product developed by Oxitec with the potential to provide benefits to human and animal health in the fight against vector-borne diseases. Regulatory oversight of products of biotechnology should be science-based and risk-proportionate in order to protect human health and the environment, and should allow timely entry into the marketplace of innovative products with potential benefits to human health and the environment." (0390 p. 1)

ADAPCO stated that:

> "ADAPCO has been a provider of mosquito control solutions to the public health vector control industry for over 35 years. During this time, we have seen an increase in the number of invasive mosquito species coming into the US. Simultaneously, the control tools available to effectively manage mosquito populations has declined. Investigation of novel and promising technologies that aid in the mission of preserving public health is critical. . . . Considering the importance of *Aedes aegypti* as a vector of arboviruses and the significant challenges associated with controlling this species (insecticide resistance, larval and adult behaviors, etc.), novel methodology for control of this species should be investigated." (0391 p. 1)

Andrea Leal, Executive Director of the Florida Keys Mosquito Control District, stated that:

> "Because of the difficulty in controlling Aedes aegypti and increase in resistance to commonly-used pesticides, we are constantly looking for new tools to add to our toolbox. This particular product could have a major impact on the mosquito control community as well as aid in the control of this widespread vector as demonstrated during similar studies in South America." (0422 p. 1)

2. <u>Role OX5034 could play in integrated vector management</u>

Five comments (0365-1, 0365-2, 0367, 0391, 0393) described how OX5034 could be used in Integrated Vector Management.

Mustapha Debboun, General Manager of Delta Mosquito & Vector Control District in Visalia California, stated that:

> ". . . as one of the General Managers of the Mosquito & Vector Control Districts, I am very interested in the use of Sterile Insect Technique (SIT) such as Oxitec's non-biting male Friendly Ae. aegypti as an augmented Integrated Vector Management (IVM) tool to safeguard public health and protect personnel from the serious viruses they transmit, i.e., Dengue, Chikungunya, Yellow fever, and Zika." (0367 p.1)

District and General Managers of several California mosquito and vector control agencies, voicing support for amending and extending the EUP, stated that:

> "Our mosquito control districts use an Integrated Vector Management approach, an evidence-based, data-driven decision-making process, to suppress mosquitoes and mosquito-borne diseases and protect public health. There is a critical need for new and innovative mosquito control strategies, including Sterile Insect Techniques, that could be integrated into our vector management programs. We are very interested in exploring the efficacy and control potential of

> Oxitec's non-biting male Friendly™ *Aedes aegypti* mosquitoes to determine if this method might be a viable option for use in controlling *Aedes aegypti* in California." (0365-2 p. 2)

Several entities involved in mosquito control efforts, American Mosquito Control Association, Anastacia Mosquito Control District, Delta Mosquito and Vector Control District, Entomological Society of America, Mosquito and Vector Control Association of California, Northwest Mosquito and Vector Control Association, Society of Vector Ecology, stated that:

> "Innovative mosquito control interventions are needed to complement the full suite of Integrated Vector Management tools. There are many different factors that play into identifying the appropriate public health intervention based on disease risk and as such mosquito control agencies need a variety of tools to effectively control disease-transmitting mosquitoes." (0393 p. 1)

The Mosquito and Vector Control Association of California (MVCAC), comprised of approximately 70 California mosquito control and public health agencies, stated that:

> "Many MVCAC members are exploring the use of Sterile Insect Techniques to complement other control methods to reduce the population of disease-transmitting mosquitoes. As such, we encourage the EPA to approve Oxitec's amendment to its Experimental Use Permit (EUP) to bring its Friendly™ *Aedes aegypti* mosquitoes to California." (0365-1 p. 1)

ADAPCO stated that:

> "Within the mosquito control industry, Integrated Mosquito Management (IMM) is the foundation of a well-balanced program. Briefly, this entails using a combination of practices to effectively prevent and control mosquitoes. This includes surveillance of mosquito populations, physical control (i.e. removing or augmenting larval habitats), biological control (i.e. mosquito fish to eat larvae), and the application of larvicides and adulticides. Oxitec's Friendly™ *Aedes aegypti* mosquitoes represent a novel way to combat mosquito populations." (0391 p. 1)

3. <u>Potential environmental benefits of OX5034</u>

Some comments (0377, 0385, 0386, 0403, 0421, 0427, 0437, 0459) described potential benefits OX5034 might provide to the environment.

Mark van der List stated that, as a beekeeper:

> "I support innovative mosquito control techniques that are environmentally sustainable, such as Oxitec's non-biting male Friendly™ *Aedes aegypti* mosquitoes. Self-limiting mosquitoes work by finding and mating with invasive *Aedes aegypti* females and the suppression effect is specifically

> targeted to this species of mosquito. This specificity is critical as it leaves pollinators, such as bees and butterflies, unharmed." (0386 p. 1)

The Founder of Marion Wilson Butterfly Garden stated that:

> "As an environmentalist and endangered butterfly conservation advocate, protecting our wildlife is one of the biggest gifts we can give to this world. As the founder of the Marian Wilson Butterfly Garden in Hermosa Beach California, I believe that Oxitec's science-based mosquito control methods will be extremely beneficial in preserving our local ecosystems and wildlife. With the immensive [sic] rapid urbanization and infrastructural development in big cities such as Los Angeles, San Diego, and San Francisco, native butterflies such as the El Segundo Blue have gone endangered and almost extinct. In addition to the rapid industrialization, invasive pests have also disrupted the chain and have pushed native insects to the point of extinction. With Oxitec's leading technology, it will be possible to control and monitor the amount of invasive pests and help reduce competition against our native species. I support efforts to reduce invasive species in an environmentally-sustainable way. Having innovative mosquito control techniques in California that protect the health of butterflies is very important and I appreciate the environmental benefits of Oxitec's technology." (0403 p. 1)

Lawrence Hribar stated that:

> "Should this work be successful it will result in reduced pesticide use and reduction of numbers of an exotic introduced pest mosquito species." (0421 p. 1)

Anonymous (0437) stated that:

> "Expanding the testing could benefit us so insects do not harm our environment. While it is good to use pesticides to get rid of insects, some chemicals and ingredients is very harmful to us humans and the environment. To change this, try experimenting environment-friendly chemicals and ingredients so it can kill the insects, but not the environment." (0437 p. 1)

The Florida Mosquito Control Association and the Florida Mosquito Control Foundation further stated that:

> "Genetically modified organisms have successfully been used in agriculture for decades, including applications in/near/over residential areas with no unacceptable adverse impacts." (0385 p. 1)

Michael Weissmann, Ph. D. Entomology, Kallima Consultants, stated that:

> "By targeting this mosquito directly, there is no impact on other species, including native mosquitoes and other insects." (0459 p. 1)

Julian Morris, the Reason Foundation, stated that:

> "The evidence indicates that there is unlikely to be any significant adverse impact on the environment or human health. To the contrary, the reduction in use of pesticides is likely to benefit the environment, while the greater efficacy and efficiency of this mosquito control method is likely to benefit human health." (0377 p. 7)

E. Comments Describing Steps Taken to Ensure Testing is Conducted Safely

Four comments (0377, 0381, 0389, 0415) cited the steps being taken to ensure the testing will be safely conducted.

The American Mosquito Control Association (AMCA) stated that:

> "Like all pesticides, Oxitec's genetically modified mosquitoes are regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). To be approved for an EUP or registration under FIFRA, pesticides must be shown to not cause unreasonable adverse effects to humans or the environment." (0381 & 0389 p. 1)

The American Mosquito Control Association (AMCA) added that:

> "AMCA also understands the proposed amendment to the EUP will continue significant protections, including weekly monitoring and sampling of the mosquito population in the treatment areas, enabling the Agency to determine the product meets the FIFRA safety standard. Additionally, it's important to note that only the adult male self-limiting mosquitoes will be present in the environment and they do not bite people, further supporting EPA's finding that the mosquitoes are not expected to present risks to human health. We also concur and applaud that if an unforeseen event occurs, EPA will have the right to cancel the EUP at any point during the extended 24-month period." (0381 & 0389 p. 1-2)

Julian Morris, the Reason Foundation, stated that:

> "In OX5034, the tTAV gene is *expressed* only in female offspring, through the use of sex-specific alternative splicing. The use of tTAV enables efficient production of OX5034 because the self-limiting gene can be switched off by exposing females to tetracycline. But due to the use of sex-specific alternative splicing, males are not dependent on tetracycline. Indeed, eggs for release have never been exposed to any tetracycline (only the female parents have)." (0377 p. 7) [Footnote Omitted]

Leor Kaminsky voiced the opinion that the impact of OX5034 on human health or the environment would be negligible based on several considerations, arguing that OX5034:

> ". . . will only affect the targeted mosquito species and only the female offspring will be killed . . . . the probability that genetically modified mosquitos will affect humans or animals is negligible since the mosquitos released will be male and male mosquitos do not bite humans or animals . . . Ae. aegypti are a uniquely peri-domestic species adapted to living in areas populated by humans and therefore, any population change among Ae. Aegypti would have very little impact on ecosystem health . . . ." (0415 p. 1)

Leor Kaminsky also pointed out that EPA had evaluated OX5034 when determining whether an EUP could be granted in 2020 for testing of OX5034:

> "The EPA evaluated the risk of the genetically modified mosquitos increasing the ability of wild mosquitos in the release area 'to vector/transmit disease, result in larger population numbers or result in more robust mosquitos' and found that this impact is unlikely (Striegel and Pierce, 2020, pg. 6). Additionally, the EPA evaluated the risk of these genetically modified mosquitos affecting non-target organisms such as bats and amphibians and found that there is no expected 'direct adverse effects due to consumption' on these non-target organisms based on 'acute oral toxicity studies and bioinformatics analyses,' (Striegel and Pierce, 2020, pg. 6)." (0415 p. 1)

The American Mosquito Control Association (AMCA) stated that:

> "AMCA also understands the proposed amendment to the EUP will continue significant protections, including weekly monitoring and sampling of the mosquito population in the treatment areas, enabling the Agency to determine the product meets the FIFRA safety standard. Additionally, it's important to note that only the adult male self-limiting mosquitoes will be present in the environment and they do not bite people, further supporting EPA's finding that the mosquitoes are not expected to present risks to human health." (0381 & 0389 p. 1)

F. Position of Agencies and Others Tasked with Mosquito and Vector Control

Comments in this category (0365-1, 0365-2, 0374, 0375, 0392, 0393, 0422) described the support for and willingness of vector control agencies and others responsible for controlling *Ae. aegypti* mosquito to participate in evaluating whether the OX5034 mosquito would be a useful tool in *Ae. aegypti* mosquito control strategies.

The Mosquito and Vector Control Association of California (MVCAC), comprised of approximately 70 California mosquito control and public health agencies, stated that:

> "As California mosquito agencies seek additional effective and innovative control strategies that can be used as part of an Integrated Vector Management approach, we welcome the opportunity to evaluate Oxitec's technology and determine if it is an efficacious mosquito control tool to protect public health." (0365-1 p. 2)

The Mosquito and Vector Control Association of California (MVCAC), comprised of approximately 70 California mosquito control and public health agencies, stated that:

> "A number of MVCAC's member agencies have expressed interest in evaluating Oxitec's non-biting male Friendly™ *Aedes aegypti* mosquitoes as a potential way to safeguard public health. We are encouraged that the EPA approved Oxitec's technology for a pilot project in the Florida Keys and by extending the EUP, mosquito control agencies in California will have the ability to assess this emerging technology to determine the control potential in local communities." (0365-1 p. 1)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD), explained that:

> "During the process that led us to this point, there had been numerous concerns raised from citizens and environmental groups about the program. Each concern was evaluated by the EPA and answered in writing. It was determined by the EPA that none of the concerns posed any unreasonable risk to humans, animals (including endangered species), or the environment in the Florid Keys. These findings are also confirmed by nine Florida State agencies agreeing there would be no expected impacts. These agencies, which include FDACS, the Florida Department of Health, Florida Department of Environmental Protection, and Florida Fish and Wildlife, regulate all mosquito control activities at the state level. Our local health department is very supportive of this project." (0374 p .1)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District (FKMCD), stated that:

> "Oxitec has been an excellent and dependable partner in this venture. Their scientists know their technology well and they are there at every step of the process. I hope the EPA will approve the expansion of this EUP to include California so they can identify other advantages to be gained from the Oxitec Project. This can lead to improved public health possibilities over a disease vector that has plagued mankind for several millennia." (0374 p. 2)

District and General Managers of several California mosquito and vector control agencies (0365-2), voicing support for amending and extending the EUP, stated that:

> "We are encouraged by earlier results from Oxitec's release of Friendly™ *Aedes aegypti* mosquitoes in the State of São Paulo, Brazil in 2019. The potential to reduce populations of *Aedes aegypti* without the use of conventional pesticides has enormous advantages and we encourage the EPA to approve Oxitec's EUP amendment to expand the use of its technology to California." (0365-2 p. 2)

Several entities involved in mosquito control efforts, American Mosquito Control Association, Anastacia Mosquito Control District, Delta Mosquito and Vector Control District, Entomological Society of America, Mosquito and Vector Control Association of California, Northwest Mosquito and Vector Control Association, Society of Vector Ecology, stated that:

> "Collectively, we are charged with protecting millions of residents from mosquito-transmitted diseases and we believe that it is necessary and timely to extend testing of the OX 5034 *Aedes aegypt*i mosquitoes to California. Mosquito control and public health experts face increasing challenges in controlling disease vectors, especially invasive *Aedes aegypt*i mosquitoes which pose a significant public health threat." (0393 p. 1)

Andrea Leal, Executive Director of the Florida Keys Mosquito Control District, stated that:

> "We continue to have community support for this project, as recorded in a county-wide referendum in 2016. Additionally, we have had state approvals for our current project by the Department of Agriculture and Consumer Services as well as our local board of commissioners." (0422 p. 1)

Dan Killingsworth, Director of Operations for Environmental Security Pest Control, stated that:

> "I recognize the value of the Oxitec *Aedes aegypti* non-biting male mosquito release program and advocate for the approval of an Oxitec EUP to begin this work in the state of California. This method will be an essential tool for the mosquito control districts in California who are battling to manage and overcome the daunting challenges presented by the *Aedes aegypti* mosquito." (0392 p. 1)

Norman Leppla, Integrated Pest Management University of Florida, voicing "support for Oxitec's application to amend it Experimental Use Permit (EUP) to pilot the release of its *Aedes aegypti* mosquitoes in California," stated that:

> "I support Oxitec's efforts to pilot its technology in California and encourage the EPA to provide mosquito and vector districts in California with the opportunity to work with Oxitec to evaluate the control potential of its technology in local communities." (0375 p. 2)

EPA Response to Comments in 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. *Ae. aegypti* mosquitoes vector several deadly viral diseases, including dengue, that infects millions of people and likely kills between 10,000 and 20,000 humans globally per year.[1] *Ae. aegypti* is widespread in the southern US,[2] and as the climate warms *Ae. aegypti* is likely to extend that range. Multiple incidences of disease caused by dengue, chikungunya and Zika viruses have been reported in the US over the past 20 years as described in comment. Dengue,[3] chikungunya,[4] and Zika[5] viruses continue to circulate in the Caribbean and Mexico, constituting a continuous threat to the southern United States, where *Ae. aegypti* occurs. Zika virus, for example, may reappear at any time.[6] In addition to the behavior of *Ae. aegypti* that makes control difficult, e.g., they are peri-domestic, day-fliers and they lay their eggs in small, cryptic sources, resistance to insecticides has been documented in *Ae. aegypti* mosquito populations, including to sprayed insecticides and the larvicides used to manage breeding sites, making control efforts ever more difficult.[7] Commenters noted that currently at best mosquito control districts are controlling only 30 to 50% of *Ae. aegypti* mosquitoes in their districts using currently available methods. 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.[8]

EPA has taken these comments into consideration in its deliberations on whether to extend and amend the EUP allowing limited testing of OX5034 under FIFRA section 5 EUP provisions. EPA has also taken into consideration that an EUP, extended and amended, 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 that sufficient regulatory controls are in place to prevent unreasonable adverse effects on health and the environment.

[1] Shepard DS, Undurraga EA, Halasa YA, Stanaway JD (August 2016). "The global economic burden of dengue: a systematic analysis". The Lancet. Infectious Diseases. 16 (8): 935–41; Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6:e1760; Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL et.al. The global distribution and burden of dengue. Nature;496:504-507.
[2] https://www.cdc.gov/mosquitoes/mosquito-control/professionals/range.html
[3] https://www.paho.org/en/news/23-11-2020-caribbean-told-unite-control-dengue-during-covid-19-pandemic
[4] Mowatt, L and S.T. Jackson. 2014. Chikungunya in the Caribbean: An Epidemic in the Making. Infect Dis Ther (2014) 3:63–68 DOI 10.1007/s40121-014-0043-9
[5] https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0006007
[6] https://asm.org/Articles/2018/September/Where-Did-Zika-Go-And-Will-It-Come-Back
[7] McGregor, B.L. and Connelly, C.R., 2021. A review of the control of Aedes aegypti (Diptera: Culicidae) in the continental United States. *Journal of medical entomology*, *58*(1), pp.10-25.
[8] https://www.cdc.gov/parasites/dirofilariasis/faqs.html

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

Comments (0370, 0380, 0405) in this category expressed concern that biting OX5034 females might be present in the test areas. Comments suggested mechanisms through which commenters believe that biting *Ae. aegypti* females carrying the OX5034 transgenes could be present in the test area. Commenters' suggestions on routes through which this might occur included: (1) contamination of OX5034 adult male releases by adult OX5034 biting females or incomplete penetrance of the OX5034 trait; (2) sufficient tetracycline in the environment to allow OX5034 females to mature to adults; and (3) emergence of resistance.

A. Comments Questioning the Penetrance of the OX5034 Trait

Several comments (0394, 0397, 0460) expressed concern that *Ae. aegypti* females carrying OX5034 genes, i.e., mosquitoes that bite, may be present in the test areas.

Friends of the Earth referred to the draft Environmental Assessment conducted by FDA on OX513A, Oxitec's first-generation transgenic mosquito and not here at issue, stating that:

> "Oxitec's initial Draft Environmental Assessment (EA) to the Food and Drug Administration (FDA) acknowledges that it is inevitable that some biting female GE mosquitoes will be released. The sorting is conducted by hand and could result in up to 0.5 percent of the released insects being female. If 100 million mosquitoes were released, 0.5 percent could mean that an additional 500,000 biting mosquitoes could be present in the environment. However, checks by the Mosquito Research and Control Unit (MRCU) in the Cayman Islands on one production batch on May 12, 2017 revealed 9 females in one release pot of 500 (1.8%), nine times the agreed level. If the sorting of GE mosquitoes for the field trials were to have similar results as the Cayman Islands, millions of GE female mosquitoes, which can bite and transmit disease, could be released into the environment during the experiments." (0394 p. 4) [Footnotes Omitted]

Comment 0460 representing a mass mail campaign stated that:

> "Oxitec claims biting females will not be released. A peer-reviewed study proved them wrong." (0460 p. 2)

GMO Free Florida stated that:

> "Even in the absence of tetracyclines Oxitec's mosquitoes are likely to remain in the environment due to the offspring that survive to adulthood without exposure to tetracycline.

> The Food and Agriculture Organization of the United Nations agrees on their website stating, *"The transgenic approaches instead can have potentially unforeseen consequences because the released insects are not sterile and therefore will reproduce and become established.*" (0396 p. 9; 0397 pp. 7, 13, & 21) [Footnote Omitted] [Emphasis in Original]

Two comments (0397, 0400) questioned the sample size used to determine the penetrance of the trait.

Florida Keys Environmental Coalition stated that:

> " . . .this vendor claiming they tested 500 eggs and no females were produced, with 500 being accepted by this EPA as a statistically significant sample size when releasing, or being approved to release, over 1 billion GM mosquitoes, or 2 billion eggs. Without an equation that proves this is correct, it is but an arbitrary number. Over the last year since the approval, Oxitec has commented that they have tested vast quantities more in field trials in Brazil with similar results, but there is not data and this is but an unsupported claim. It also assumes that their trapping is comprehensive[...] The EPA should ask Oxitec how it does statistical math on field performance data." (0400 p. 8)

GMO Free Florida stated that "[S]mall samples of hundreds or thousands of mosquitoes cannot be extrapolated to draw conclusions that no females will be released when millions of OX5034 are released," stating that:

> "Oxitec's previous studies have been too small to determine that no females will be released. Data from Brazil is not sufficient. . . . Testing should be done in the laboratory using numbers of mosquitoes equivalent or greater to the amount planned for use in the Florida Keys and California to determine that no females will survive to adulthood in these test areas. Small samples of hundreds or thousands of mosquitoes cannot be extrapolated to draw conclusions that no females will be released when millions of OX5034 are released. Since traps do not capture all *Aedes aegypti*, data from traps used in previous releases in Brazil are not sufficient to determine no females survived to adulthood in the Brazilian experiments or will not survive to adulthood in the Florida Keys or California experiments, especially considering the large number of OX5034 that will be released. . . . Since traps do not capture all *Aedes aegypti*, data from traps used in previous releases in Brazil are not sufficient to determine no females survived to adulthood in the Brazilian experiments or will not survive to adulthood in the Florida Keys or California experiments, especially considering the large number of OX5034 that will be released." (0397 p. 20)

One comment (0400) requested that reliability of the fluorescence marker be tested, stating:

> "Why is there no resiliency testing of the fluorescent marker? The only people who benefit from the markers not being reliable is Oxitec. It would seem prudent to have some reasonable level of

testing, given the public would like to know if there are biting females produced at any generational level once the releases begin." (0400 p. 8)

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

Comments (0383, 0388, 0394, 0396, 0397, 0401, 0432) argued that tetracycline is a commonly used antibiotic, and that it is possible that OX5034 mosquito larvae might encounter tetracycline at concentrations in the environment that are high enough to allow the female larvae to mature into adulthood. Comments suggested three potential routes of exposure to tetracyclines, through agricultural production use, in sewage, or in food production and animal husbandry.

Two comments (0383, 0397) stated that tetracycline(s) are expected to be found in the environment.

GeneWatch UK argued that:

> " . . . , even if GE females are not released, the finding on lack of exposure to biting females depends on the assumption that females do not survive to adulthood. In reality, survival may occur if resistance develops or because of environmental exposure to tetracycline (see more detail in our previous submission)." (0383 p. 5) [Footnote Omitted]

GMO Free Florida stated that:

> "Since tetracyclines are also found in the environment, there are other ways OX5034 x wild *Aedes aegypti* larvae could be exposed such as tetracycline being found in sewage, drinking water, animal feces, liquid manure and even tetracycline applied as a veterinary medicine in the water bowls of livestock and pets placed indoors or outdoors. … Since tetracycline is not likely to be eliminated from pet food or the environment in the near future, there is a significant chance that these Oxitec mosquitoes will survive and therefore biological containment is an impossibility at this point." (0397 pp. 18 & 21)

1. <u>Agricultural production</u>

Friends of the Earth stated that:

> " . . . tetracycline is a common antibiotic used in agricultural production. 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. California has massive agricultural regions, and it is necessary to look at levels of tetracycline use in each of the counties targeted for release and to compare this with the levels of tetracycline in the environment that could impact the survival of female GE mosquitoes. EPA has redacted

> information about tetracycline levels from Oxitec's proposal, however, so it is not possible to assess this potential risk. 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." (0394 p. 3)

The Center for Food Safety stated that:

> "Tetracycline, the genetically engineered kill switch for the female mosquitoes, may not work to keep the female mosquitoes from surviving if they are released. In both Florida and California, tetracycline is sprayed on citrus and found in abundance in waste water treatment plants and Concentrated Animal Feeding Operations." (0396 p. 5)

GMO Free Florida argued that while "Oxitec uses a diet supplemented with 30 µg/ml of the tetracycline to rear its mosquitoes in the lab. The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow the GE mosquitoes to breed" because:

> "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 is likely to be more than enough to inactivate the killing mechanism." (0397 p. 18) [Footnotes Omitted]

Californians for Pesticide Reform stated that:

> "Nor does the docket discuss the significant agricultural lands in California and the associated chemicals used in these settings, such as tetracycline, that would impact any field trial." (0401 p. 1)

Christopher Lish similarly stated that:

> "The docket does not discuss the significant agricultural lands in California and the associated chemicals used in these settings, such as tetracycline, that would impact any field trial." (0388 p. 1)

2. <u>In Sewage</u>

Friends of the Earth stated that:

> " . . .tetracycline is also a prevalent compound found in sewage due to contamination from agricultural run-off and consumer disposal. Aedes aegypti may be found to breed in sewage treatment plants, septic tanks, and cesspits in the Florida Keys and in California." (0394 p. 3)

GMO Free Florida, rebutting Oxitec's statement that they were unable to find levels in sewage in the scientific literature throughout the world needed for female OX5034 to have high survival rates, stated that:

> ". . . , studies do exist suggesting this level exists in slurry samples, tetracycline at these levels are in some municipal sewage, and prescribed doses of tetracycline for medicating animal drinking water." (0397 p. 18) [Footnote Omitted]

3. Food, Food Waste and Animal Husbandry

One comment (0397) argued the possibility of food, food waste and animal husbandry being a source of tetracycline in the environment.

In response to EPA's conclusion on the implausibility of cat food being a source of tetracycline exposure, GMO Free Florida argued that:

> " . . . the conditions would in fact be common in the Florida Keys and parts of California. Water is often added to pet food and the Florida Keys, and parts of California, are often areas that are only used temporarily throughout the year, such as only in the fall and winter as vacation homes. As this is a common occurrence, it is often common for people to leave vacation homes without cleaning up pet dishes. This would allow for the right amount of time for *Aedes aegypti* to deposit eggs and those mosquitoes survive to adulthood. Peer reviewed evidence indicates that *Aedes aegypti* often deposit eggs indoors in homes. In fact, peer reviewed evidence, *"found that the infestation of indoor containers by Ae. aegypti was greater than outdoor containers.*" (0397 pp. 17-18) [Footnotes Omitted]

And:

> "Since as much as 18.4% of OX513A offspring could survive when exposed to pet food, which they are exposed to in the Florida Keys and California, it is extremely unlikely that the OX5034 female mosquitoes will all die, and extremely likely that the Oxitec mosquitoes will survive beyond the duration of the trial. . . . An employee of the Florida Keys Mosquito Control District admitted that Aedes aegypti larvae have been found in pet dishes, suggesting this scenario would likely occur if released in the Keys or California" (0397 pp. 13 & 17) [Footnote Omitted]

GMO Free Florida claimed that husbandry of domestic animals in general could be sources of tetracyclines:

> " . . . the Florida Keys has a large chicken population located in the urban areas. Pets also exist in urban areas and since pets are often fed foods containing tetracyclines their feces will also

> contain tetracyclines. Manures such as chicken or cow manures will also be used as fertilizers in these areas." (0397 p. 18) [Footnote Omitted]

And:

> ". . . , a study suggests that it can take 5 months for tetracyclines to degrade just 50% in liquid manure. Oxitec also neglected to realize that most people will likely put drinking water medicated with tetracycline intended for their animals, in shaded areas like a porch, and it is believed shaded areas are where *Aedes aegypti* most often place their eggs. Peer reviewed evidence also indicates that *Aedes aegypti* often deposit eggs indoors in homes as well where the temperature is controlled and sunlight isn't a factor." (0397 pp. 17-18) [Footnotes Omitted]

And:

> ". . . . overwhelming peer reviewed evidence suggests *Aedes aegypti* are equally or more attracted to water with certain materials in it than they are clean water. These include containers with algae, decaying leaves, infusions of various leaves and grasses, even horse manure and sewage water. Studies also suggest *Aedes aegypti* larvae can develop in septic tanks, sewage treatment plants and cesspits." (0397 p. 18) [Footnotes Omitted]

C. Comments on the Potential Emergence of Resistance to Arise to the OX5034 Traits

Two comments (0383, 0397) were concerned about resistance arising through mutations in the tTAV-OX5034 transgene or through factors of the genetic background of *Ae. aegypti* populations. One comment stated that no testing to determine the rate at which the emergence of resistance to the transgenes could arise had been performed.

One comment (0397) argued that resistance to the tTAV-OX5034 female-lethal mechanism can be expected to arise based on the numbers of OX5034 released and the expected mutation rate.

GMO Free Florida argued that "according to the mutation rates," Oxitec's previous studies had been too small to determine that no females had been released:

> ". . . the percentage of surviving GM insects, including biting females, could also increase if resistance to the genetic killing mechanism evolves over time: for example, genetic mutations in the insects which allow the GM insects to survive and breed successfully could be rapidly selected for during mass production." (0397 pp. 6 & 20) [Footnotes Omitted]

GeneWatch UK stated:

> " . . . EPA has not assessed whether resistance will develop and therefore has made no assessment of whether GE females will survive in the longer term. It states in the Response to Comments: "*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.*" (p.16, EPA-HQ-OPP-2019-0274-0355). Thus, there is no basis to conclude that exposure to biting females will be low." (0383 p. 6) [Emphasis in Original]

EPA Response to Comments in Unit IV.A - Comments Questioning the Penetrance of the OX5034 Trait.
Regarding the comment on the draft Environmental Assessment conducted by the FDA and the information from the Cayman Islands, both reports cover "OX513A," which is a genetically engineered mosquito product not at issue for this EUP amendment and extension. Given the reference to the manuals sorting efficiency, however, it is relevant to note that OX5034 does not rely on manual sorting of the males from the females prior to environmental releases and thus, the considerations raised by the comment are not relevant to this current action. Specifically, the genetic construct integrated into OX5034 mosquitoes genetically separates males from females (i.e., by not allowing females to survive into adulthood), which obviates the need for manual separation of the sexes.

Regarding the two comments contending that OX5034 females may be present in the environment, insufficient information was provided to enable a response by EPA. The first commenter did not provide a reference to the claim that a "peer-reviewed study proved them wrong." Regarding the second comment on the "potential for unforeseen consequences because the released insects are not sterile and therefore will reproduce and become established," that statement by the FAO was not made in reference to OX5034 or any other specific transgenic organism, mosquito or otherwise, and the website does not provide any additional information that would establish the relevance of the statement for OX5034.

Regarding the two comments questioning whether the sample size used was adequate to detect the penetrance of the trait, as discussed in the risk assessment[9] of the original EUP, both laboratory and field studies using homozygous as well has hemizygous females of the same as well as different genetic backgrounds has demonstrated the complete penetrance of the female-lethal trait in the absence of the antidote in the larval rearing medium. With respect to the number of mosquitoes tested, one commenter specifically mentioned that EPA's assessment was based on the evaluation of 500 homozygous OX5034 females. While it is true that penetrance in 500 homozygous OX5034 females was evaluated, in the same experiment an additional 1,000 hemizygous females (representing the genetic composition of individuals in the field) were also assessed (see EPA's 2020 risk assessment Unit II.B.3.a; Docket number: EPA-HQ-OPP-2019-0274-0359). In all instances, penetrance of the female-lethal trait

[9] The term "risk assessment" hereinafter refers to Human Health and Environmental Risk Assessments performed for OX5034 containing the Tetracycline Repressible Transactivator protein variant (tTAV-OX5034) protein, a DsRed2 protein variant (DsRed2-OX5034) and the genetic material necessary for their production in OX5034 *Ae. aegypti*. The assessments are available in docket EPA-HQ-OPP-2019-0274.

was 100%. In addition to these 1,500 individuals, Oxitec conducted another penetrance study on mosquitoes that carried the OX5034 traits, this time the individuals were sampled directly from field releases in Brazil and thus carried the local mosquito background genetics. As before, that study also found complete penetrance of the OX5034 phenotype.

EPA Response to Comments in Unit IV.B. - Comments Questioning Whether Sufficient Tetracycline Occurs in the Test Environment to Allow Tetracycline-Dependent Female *Ae. aegypti* Mosquitoes to Mature to Adults. Regarding the comments on potential environmental tetracycline sources, for this EUP amendment and extension, as well as the original OX5034 risk assessment, levels that were demonstrated to be able to rescue the female-lethal phenotype were compared to concentrations of the tetracycline analogues typically found in the environment as a result of industrial or household tetracycline usage. As before, a literature search was conducted that focused on potential environmental sources that are present in the United States, as those were deemed to be the most relevant to the EUP. In all cases the minimum concentration for each tetracycline analogue required to rescue OX5034 females capable of maintaining flight is higher than the mean concentrations found in environmental water bodies for the studies reviewed. The EPA also considered potential sources of tetracycline specific to the proposed trial areas. Here, EPA worked closely with the Florida Department of Agriculture and Consumer Services and the California Department of Pesticide Regulation regarding the use of tetracyclines in the trial areas. After careful consideration of all available data EPA concluded that the likelihood that OX5034 mosquitoes would encounter tetracycline levels high enough to result in OX5034 females is low. The 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; 2) commercial citrus, apple, pear, nectarine, and peach crops; 3) and commercial cattle, poultry, and pig livestock facilities) by limiting proximity of the outer trial boundaries to such potential sources, the likelihood will be reduced to negligible levels. For further discussion on limiting the proximity of the outer trial boundaries to potential tetracycline sources, EPA refers the commenter to Unit VIII of this document.

Regarding the comments on agricultural sources of tetracycline, EPA found that commercial agricultural areas (i.e., livestock and certain crops) are unlikely to pose a potential source of environmental tetracyclines that could lead to the survival of OX5034 into adulthood, but that those sites are likely to contain the highest levels of environmental tetracycline. Thus, as outlined in the preceding paragraph, the Agency imposed OX5034 release restrictions under this EUP amendment and extension from the perimeter of commercial citrus, apple, pear, nectarine, and peach crops as well as commercial cattle, poultry, and pig livestock facilities to further reduce the likelihood that OX5034 mosquitoes could encounter increased levels of tetracycline as a result of these applications. In its decision to restrict the release areas around agricultural livestock facilities, EPA considered the significant agricultural areas in the four CA counties proposed as trial sites.

Regarding the comments on sources of tetracycline in sewage and that *Ae. aegypti* has been found to occasionally oviposit in these sites, these considerations had previously been addressed in the Response to Comments document for the 2020 EUP (EPA-HQ-2019-0274-0355). It should also be noted that, as for the previous OX5034 EUP, the Agency is again restricting access of OX5034 to wastewater treatment facilities for the current EUP amendment and extension, i.e., imposing a 500 m buffer zone around these sites, and refers the commenter for a further discussion on these restrictions to Unit VIII (response to VIII.B) of this document.

With regard to food, food waste, and animal husbandry sources of tetracycline, EPA previously discussed this point in the Response to Comments document for the original EUP. That information can be viewed in the docket established for that action under document number EPA-HQ-OPP-2019-0274-0355. EPA concluded that in addition to requiring sufficient levels of tetracycline, several steps would need to occur for pet food to be a plausible source of OX5034 females, these were: "*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*." The same assessment is applicable to the current EUP amendment and extension for OX5034. The likelihood that pet food that is located indoors would be a source of tetracycline, as claimed by one commenter, is even less likely than pet food left outside given the restricted access that mosquitoes have to indoor spaces (see also the discussion in Unit V.B of this document on inhalation exposure). Regarding the comment on pet feces as a source of tetracyclines, EPA generally finds the scenario that pet feces could contain levels of tetracycline that are high enough to result in female OX5034 adult rescue unlikely because of the reasons outlined for the pet food above, i.e., must contain sufficient levels of tetracyclines, maintaining standing water for certain period of time, and sunlight-mediated photolysis. In addition, unlike commercial livestock facilities which are likely to have the highest levels of tetracyclines (due to the frequency and amount of antibiotics used in these facilities), the density of animals treated with tetracycline in the OX5034 test areas is expected to be significantly lower and would consist of sporadic veterinary treatment of some pets. Tetracycline treatment of feral animals would occur even less frequently.

EPA Response to Comments in Unit IV.C. - Comments on the Potential Emergence of Resistance to Arise to the OX5034 Traits. Regarding the comment that resistance can develop through mutation, the commenter cites an article published by Zhao and colleagues in 2020. EPA has carefully analyzed that publication in the context of the environmental releases of OX5034 and published its findings in the OX5034 docket (document number EPA-HQ-OPP-2019-0274-0362) under the title "*Review of the Zhao et al., 2020 study on "Genetic breakdown of a Tet-off conditional lethality system for insect population control" and its relevance to the OX5034 Ae. aegypti Experimental Use Permit; EPA File Symbol 93167-EUP-1.*" Briefly, EPA concluded that the Zhao et al. study is consistent with EPA's assessment that there

is a negligible potential for OX5034 female survival during the EUP due to genetic resistance to the lethal trait and that demonstration of offspring survival in the Zhao study does not imply that there will be survival of female OX5034 during the EUP. Similarly, this publication does not change EPA's conclusion regarding the potential for genetic resistance for this EUP amendment and extension.

Regarding the comment that resistance to the female-lethal trait could be "rapidly selected for during mass production," EPA finds that the use of tetracycline during mass rearing is likely to alleviate any selection pressures as its use allows females to develop into functioning adults, i.e., reducing the need for resistance development.

Regarding the comment that EPA has not assessed the likelihood for resistance to develop during field releases, EPA did conduct that assessment as part of the original application for the OX5034 EUP (e.g., Unit II.C.4 of the original risk assessment). The quote from EPA's 2020 Response to Comments document (EPA-HQ-OPP-2019-0274-0355) that was cited by the commenter was intended to emphasize the regulatory options that EPA has for FIFRA Section 3 registrations should Oxitec wish to seek full registration of their product. It was not intended to imply that the potential for resistance had not been evaluated in the context of the EUP.

EPA has reviewed data that confirmed the ability of fluorescent screening to determine the presence/absence of the OX5034 rDNA by performing PCR-based genotyping (see the risk assessment of the original EUP, Table 4; Docket number: EPA-HQ-OPP-2019-0274-0359). Additionally, and contrary to the commenter's statement, continued testing of the reliability of the DsRed2-OX5034 marker was a requirement under the original EUP, as discussed in the risk assessment of the original EUP in Unit II.C.4. The same requirement is also part of the EUP amendment and extension for testing in California and continued testing in Florida. See also the terms of the OX5034 EUP, which can be found in the docket EPA-HQ-OPP-2019-0274 on regulations.gov.

V. Comments on Human Health Considerations

Comments (0383, 0394, 0396, 0397, 0442, 0445, 0460) were received on human health considerations. Comments on human health revolve around the ability of OX5034 and the females with which they mate to spread diseases, including the effect of the environmental releases on *Ae. aegypti* behavior in the release areas. Comments also revolved around the potential for exposure to both OX5034 males and females through the dermal, inhalation, and oral routes of exposure. Comments also questioned whether DsRed2-OX5034 and tTAV-OX5034 could be toxic or allergenic.

A. Comments on Potential for Disease Transmission from OX5034 Mosquitoes

Comments (0383, 0394, 0396, 0397, 0442, 0460) in this category revolve around whether there are factors that may affect the vectorial capacity in OX5034 mosquitoes and their offspring, including the possibility of venereal transmission of arboviruses, changes to the physiology of mated mosquitoes

leading to altered saliva composition in females, and the potential for arboviruses to evolve. Commenters also requested that EPA conduct analyses for vectorial capacity.

1. Vectorial capacity of OX5034 mosquitoes and their offspring

Three commenters (0383, 0396, 0397) suggested scenarios in which the vectorial capacity of OX5034 or their offspring may be affected. These were: venereal transmission of certain effectors from OX5034 males to wild-type females, effects of OX5034 releases on venereal and horizontal transmission of arboviruses, changes in the composition of mosquito saliva, and the effects of use of tetracycline in the production of OX5034. One of these commenters (0397) requested that the vectorial capacity of OX5034 be tested and that data on the vertical transmission of arboviruses be made available.

One commenter (0397) suggested that OX5034 males could contribute to increases in mosquito-borne diseases in the test area through seminal fluids that are transferred from OX5034 males to wild females. The commenter also stated that the life expectancy of OX5034 males would be sufficient for venereal transmission of viruses to occur, commenting that:

> "Yet another way OX5034 mosquitoes could increase mosquito-borne diseases is if there are differences in the seminal fluids of the male genetically modified mosquitoes. When a female *Aedes aegypti* receives seminal fluid and sperm from a male this causes physiological responses in the female. These physiological responses could result in changes in host seeking behavior, blood meal size, blood digestion rate, life expectancy, re-mating behavior, egg development or fecundity. Mated female *Aedes aegypti* also may be more attracted to human odors. Any, or all, of these changes could increase mosquito-borne disease transmission. If a female mosquito lives longer, for example, she may be able to have more offspring spreading the diseases further, or if she has a change in host seeking, such as biting more people than usual, she could spread diseases more. . . . An increase in multiple host feeding could increase allergy risk and multiple host feeding may affect the spread of diseases mosquitoes carry. The receipt of seminal fluid proteins that are transferred from males to females along with sperm during copulation may cause changes in host-seeking and feeding behavior. Therefore, Oxitec must also conduct studies to determine if there are differences in seminal fluid proteins and sperm of their GE mosquitoes compared to wild male mosquitoes in the Florida Keys and if any differences are observed what impact this has on the host-seeking and feeding behavior of wild *Aedes aegypti* females that mate with OX5034 male mosquitoes. (0397 pp. 10, 11 & 12) [Footnotes Omitted]

And:

> "As the OX5034 releases could alter dengue transmission, it should be noted that complex immune responses to the four types of dengue virus mean that a partial reduction in mosquito numbers can reduce cross-immunity to the different serotypes and increase the number of cases of the severe form of the disease, dengue haemorrhagic fever, which is more likely to be

> fatal. Success in reducing illness in young children can also mean more delayed and serious cases of dengue." (0397 p. 12) [Footnotes Omitted]

And:

> " . . . as no caged trials have been done to determine how long OX5034 survive in these environments . . . it should not be assumed that these mosquitoes will have the same life expectancy if released in the Florida Keys or California. However, for homozygous OX5034 males reared off-doxycycline there was a median survival of 24 days according to data Oxitec presented to the EPA. Hemizygous OX5034 males reared off-doxycycline had a median survival of 44 days. However, for Zika *"four-five days was enough to spread the virus in the body of the mosquito, and four-five days of mating were enough for venereal transmission to occur."* Which gives the OX5034 time to spread mosquito-borne diseases." (0397 p. 31) [Footnote Omitted] [Emphasis in Original]

GMO Free Florida also voiced concerns about the possibility that OX5034 releases could lead to an increase in the potential for mosquito-borne arbovirus by facilitating their transmission between mosquitoes.

The commenter stated that "[M]ale, and potentially female, OX5034 released may increase risk of Zika and other mosquito borne diseases" through "venereal transmission, Transovarial transmission and Male accessory gland proteins, which play a significant role in disease transmission," stating further:

> " . . . if male OX5034 mate with wild females harboring Zika virus, for example, not only can the male acquire Zika from the female through venereal transmission, but the offspring of this pair can acquire these diseases through vertical transmission. That means that not only can the OX5034 that are released spread Zika, but their male, and likely some female, offspring can spread Zika as well. Now these male offspring can harbor diseases like dengue fever and chikungunya, for example, and they could infect wild females through venereal transmission spreading these mosquito-borne diseases even more." (0397 pp. 9-10) [Footnotes Omitted]

And:

> "Even if no females are released the OX5034 mosquitoes may still increase mosquito-borne diseases. If the OX5034 mates with wild females harboring these diseases the male can then acquire diseases such as Zika from the infected female through venereal transmission. Since male *Aedes aegypti* can mate as many as 6 times per day, releasing hundreds of millions of males is a really bad idea as this could cause a rapid spread of a disease like ZIKV." (0397 p. 14) [Footnotes Omitted]

And:

> "Mathematically, if there are only a few Zika infected females in the wild in Florida or California, the smaller the number of males in the wild the less likely one of those males will find one of those infected females and mate. As large numbers of additional males are released it dramatically increases the odds of some of these males finding these infected females and becoming infected via venereal transmission. Now these infected males potentially go out and mate with other females spreading Zika. These females will then bite hosts, spreading Zika to humans. This will allow other wild females to acquire Zika from those infected humans via bite as well, and have male offspring that acquire Zika via transovarial transmission and then spread it via venereal transmission. Wild females that mate with wild males will also be able to bite these infected humans and have male and female offspring able to spread Zika. Now as the mosquitoes start to decrease in population, provided this experiment works as intended, there will be more Zika infected females than at the start." (0397 p. 11)

GMO Free Florida also suggested that mating of female OX5034 or wild females that mate with OX5034 males could increase disease transmission by changing the composition of the female saliva, stating that:

> *"Aedes aegypti* saliva is composed of numerous proteins which can play a role in disease transmission. For example, the protein, "aegyptin," has been found in decreased abundance in the saliva of *Aedes aegypti* infected with dengue. Changes in the levels of proteins in saliva of female OX5034 due to the transformation process, or changes in the saliva of wild females that mate with OX5034 males could cause an increase in disease transmission." (0397 p. 10) [Footnotes Omitted]

Three comments (0383, 0396, 0397) suggested the possibility that the use of tetracycline in the production of OX5034 in the UK may affect the vectorial capacity of the OX5034 mosquitoes.

The Center for Food Safety and GeneWatch UK both stated that:

> "Additional complexity needs to be considered in the light of recent studies showing that mosquito microbiomes (which can be influenced inter-generationally by the use of antibiotics) can influence vectorial capacity." (0383 p. 6; 0396 p. 5) [Footnotes Omitted]

GMO Free Florida (0397), contending that "tetracycline use can increase the risk of tetracycline resistant bacteria, may enhance susceptibility of male, and potentially female, OX5034 to mosquito-borne diseases," argued that:

> "There is some evidence that antibiotics may increase the transmission of dengue fever by *Aedes aegypti* mosquitoes. The EPA replied to the potential of the exposure to antibiotics to increase vector competence stating, *"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"*. This, however, does not take into consideration that even if no females are released the OX5034 mosquitoes may still increase mosquito-borne diseases. If the OX5034 mates with wild females harboring these diseases the male can then acquire diseases such as Zika from the infected female through venereal transmission. . . . There is some evidence that antibiotics may increase the transmission of dengue fever by *Aedes aegypti* mosquitoes. This suggests that Oxitec's mosquitoes, reared on the antibiotic tetracycline, are better able to transmit dengue fever compared to wild *Aedes aegypti*." (0397 pp. 14 & 32) [Footnotes Omitted] [Emphasis in Original]

One commenter (0397) requested that testing of the vectorial capacity of OX5034 be conducted.

GMO Free Florida furthermore hypothesized that OX5034 males could have increased capacity for horizontal and vertical transmission of arboviruses and requested that vector competence be tested:

> "It is even possible that OX5034 has higher rates of venereal transmission for mosquito-borne diseases compared to wild *Aedes aegypti* in the Florida Keys, or higher rates of vertical transmission to the offspring of OX5034, but there is no way to know since there was no data in the EPA documents and no peer reviewed studies examining this issue. . . . Zika was previously present in the Florida Keys making this a serious concern. Therefore, testing must be done to determine if male OX5034 have higher vector competence via venereal transmission compared to wild male *Aedes aegypti* in the Florida Keys and California." (0397 pp. 10 & 14) [Footnote Omitted]

The commenter also argued that "Oxitec must conduct studies to assess the ability of GE mosquitoes to effectively transmit ZIKV, dengue, chikungunya, yellow fever, and all other mosquito borne diseases spread by *Aedes aegypti*, in comparison to wild *Aedes aegypti* currently found in the test area" including venereal and transovarial transmission because:

> "In studies in Senegal for example, they found conflicting evidence for the ability of *Aedes aegypti* to transmit ZIKV. Some *Aedes aegypti* were able to effectively transmit ZIKV and others were not. In another study, *Aedes aegypti* from Cape Verde and Kedougou showed the potential to transmit chikungunya in saliva but not those from Dakar. Since Oxitec plans to release millions of male GE mosquitoes, if Oxitec's mosquitoes can more effectively transmit ZIKV, dengue, chikungunya or other mosquito borne diseases, via venereal transmission, compared to the wild *Aedes aegypti* in the Florida Keys or California, this would represent a greater risk to humans in the Keys or California than wild *Aedes aegypti."* (0397 p. 31) [Footnotes Omitted]

GMO Free Florida also requested that data on the capability of OX5034 to vertically transmit mosquito-borne diseases be made available, stating that:

> "No data was presented by Oxitec for sexual transmission or vertical transmission of mosquito-borne diseases, or testing of male accessory gland proteins despite the fact that these all play an important role in the transmission of vector-borne diseases. Therefore, this [sic] data must be presented and evaluated." (0397 p. 10)

2. *Ae. aegypti* behavior

One commenter (0397) hypothesized that OX5034 releases could lead to an increase in disease incidences by affecting the behavior of *Ae. aegypti*. The commenter hypothesized that this increase could result from, amongst other things, an increased number of mated females, more females around humans, and a reduction in female mosquitoes.

GMO Free Florida (0397) stated that releases of a substantial number of male mosquitoes is expected to increase the number of mated females, which could then pass on the infection to their offspring and to other males through mating, stating that:

> "Under normal conditions there are likely females in the wild that die virgins. Although some males are very good breeders and have mastered harmonic convergence, other males are not. Also the distance needed to find a female in the environment makes mating success much less than 100%. Even in laboratory experiments under optimal conditions female insemination rates often do not reach 100% and those rates are significantly lower in large low-density field cages. This would indicate that females are even more likely to fail to find a mate in the open environment. However, by substantially increasing the number of males, as takes place during this experiment, the number of virgin females will initially decrease as more males will be available for breeding. Virgin females obviously do not pass on diseases to their mates or their offspring so there is less risk of disease spread." (0397 p. 11) [Footnote Omitted]

The commenter further posited that OX5034 releases could lead to polyandry of *Ae. aegypti* females, which could increase the spread of disease:

> ". . . as male population density increases this increases the likelihood of incomplete sperm transfer as other males will be interrupting the mating to try to mate themselves. When this happens, females tend to mate twice or more. The more a female mates the more disease spreads as well. So adding all of these extra males also increases the risk of more mated females and more females having multiple mates." (0397 p. 11) [Footnote Omitted]

Arguing that higher numbers of male mosquitoes in the test area could increase mosquito-borne diseases by increasing the number of females around humans, GMO Free Florida stated that:

> "Many studies have indicated that male *Aedes aegypti* are attracted to humans and routinely land on humans. Male *Aedes aegypti* also produce pheromones that attract other males and females. Therefore, the release of male *Aedes aegypti* will increase the number of males and females around humans. This increase in females around humans may cause an increase in mosquito-borne diseases." (0397 p. 9) [Footnotes Omitted]

GMO Free Florida also argued that the reduction in the *Ae. aegypti* population "could also increase spread of diseases through an increase in multiple host feeding," stating that:

> "A reduction in the *Aedes aegypti* population may not necessarily mean a reduction in dengue fever. Studies suggest *Aedes aegypti* are opportunistic feeders; if there is less competition, the remaining mosquitoes may feed on more people than usual. Multiple host feeding may affect the spread of diseases mosquitoes carry. This is especially important since the release of millions of additional males will likely increase the number of mated females and mated females live longer, take larger blood meals and may be more attracted to human odors which could increase the spread of mosquito-borne diseases." (0397 p. 11) [Footnotes Omitted]

3. OX513A *Ae. aegypti* and disease incidence in release areas

Three commenters (0397, 0442, 0460) pointed to reports on the incidence of disease in areas where OX513A mosquitoes (a different transgenic mosquito product developed by Oxitec and not the product at issue here) had been released.

GMO Free Florida stated that:

> " . . . a study in Brazil using a previous version of a genetically modified mosquito, OX513A, found that cities that did not use these genetically modified mosquitoes had lower rates of dengue in 2017, compared to 2015 rates, than cities that did use the OX513A." (0397 p. 9) [Footnote Omitted]

Comment 0460 representing Mass Mailer, contending that the "long-term impacts on human health and the environment are unknown," stated that:

> "Such mosquitos were released in Brazil a few years ago. It was the time and place when a large number of babies were born with encephalitis." (0460 p. 2)

S. Rigali noted that:

> "Zika started hitting Brazil hard about a year ago. Doctors there also raised the alarm about a troubling increase in birth defects, notably microcephaly, which is marked by damaged brain development and a small head. But Zika had not been known to cause any kind of birth defects

> before, and was in fact a fairly obscure virus because it didn't even make most people sick. So there were doubts about whether the birth defects in Brazil were related to Zika. Several human cell lines have been transformed, even primary human T cells using piggyBac. These findings leave us little doubt that the transposon-borne transgenes in the transgenic mosquito can transfer horizontally to human cells. The piggyBac transposon was found to induce genome wide insertion mutations disrupting many gene functions." (0442 p. 1) [Footnotes Omitted]

But also, that:

> "Important update: Since this article was written, two things have become apparent that cast doubt on the hypothesis advanced in this article as regards the Zika virus.
> First the connection between the Zika virus and Brazil's outbreak of microcephaly as set out in this article appears increasingly tenuous with the announcement on 6th February of the Colombian President, Juan Manuel Santos (reported by the Washington Post), that there is no evidence that Zika has caused any cases of microcephaly in his country. Health officials have diagnosed 3,177 pregnant women with the virus, but in no case has microcephaly been observed in foetal scans. We also note that the Melo Oliveira paper referred to below is based on an examination of just two women." (0442 p. 1)

4. <u>Comments that pathogenic viruses could evolve in response to OX5034</u>

One commenter (0394) expressed concern that the dengue virus could evolve to become more virulent in response to OX5034 releases.

Friends of the Earth 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." (0394 p. 5) [Footnote omitted]

B. Exposure Considerations

Commenters in this category (0383, 0394, 0396, 0397) raised concerns about exposure considerations and argued that EPA did not consider this adequately in its evaluations. One commenter (0383) contested EPA's conclusion that *"exposure to OX5034, tTAV-OX5034, DsRed2-OX5034 and the genetic material encoding them through the dermal, oral, pulmonary, and ocular routes of exposure is expected to be negligible".* Commenters argued that the numbers of OX5034 males present in the test area must be taken into account when evaluating exposure considerations. This would include consideration of in-home exposure. Comments on potential routes of exposure revolved around oral, dermal, and inhalation routes of exposure as well as potential exposure to proteins in the saliva of any biting OX5034 females.

Some commenters (0383, 0394, 0396, 0397) suggested that humans could be exposed to the tTAV-OX5034 and/or DsRed2-OX5034 proteins engineered into the OX5034 mosquitoes through the oral route, i.e., through ingestion of either OX5034 larvae or adults.

GeneWatch UK stated that:

> "Ingestion may also be a potential exposure route for humans or animals, as females are expected to die at the larval stage in the water where they breed. Further, GE males are expected to be released in large numbers and to survive in each generation and could be swallowed by humans or animals." (0383 p. 5)

GeneWatch UK noted that part of the "section on allergenicity is blacked out" and does not describe the considerations leading to EPA's conclusion that:

> *" . . . exposure to OX5034, tTAV-OX5034, DsRed2-OX5034 and the genetic material encoding them through the dermal, oral, pulmonary, and ocular routes of exposure is expected to be negligible. Therefore, overall the waiver requests for the acute oral toxicity, acute inhalation toxicity, acute dermal toxicity, primary eye irritation, and primary dermal irritation are acceptable.*" (p. 23/24, EPA-HQ-OPP-2019-0274-0359)." (0383 p. 5) [Emphasis in Original]

Friends of the Earth stated that:

> "The experimental release of *Aedes aegypti* raises serious concerns about possible negative impacts on public health. 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." The risks of ingestion, whether intentional or unintentional, of GE mosquitoes by mammals, reptiles, birds, or other organisms, have not been adequately assessed." (0394 p. 4) [Footnote omitted]

The Center for Food Safety stated that:

> "A young child might even drink from a cup left inside or outside with OX5034 x wild *Aedes aegypti* larvae in it." (0396 p. 7)

GMO Free Florida stated that:

> "Oxitec appears unaware that gene transfer to intestinal microbiota from food has been observed from the consumption of GE food even in humans. . . . Gene transfer to intestinal

> microbiota from food has been observed from the consumption of GE food in humans and such a risk also exists for Oxitec's mosquitoes if swallowed." (0397 pp. 27 & 33) [Footnotes omitted]

Some commenters (0383, 0394, 0396) suggested that humans could be exposed to the tTAV-OX5034 and/or DsRed2-OX5034 proteins engineered into the OX5034 mosquitoes through the dermal route.

Disagreeing with EPA's conclusion in the risk assessment that "*the frequency of human interaction with male mosquitoes is expected to be minimal"* because males would not be expected to enter the house, GMO Free Florida argued that:

> "Dermal exposure will be frequent because male *Aedes aegypti* are attracted to humans so frequently land on humans and they produce pheromones that attract other males and females. Females carrying sperm from OX5034 males will also land on humans. . . . *Aedes aegypti* females often mate and store male sperm in the spermatheca for life. Therefore, if these novel proteins are present in the OX5034 sperm, every female that mates with a male OX5034 will be carrying these proteins and females continuously interact with humans throughout their life. The question of whether or not these novel proteins, or any alteration in proteins that may increase allergies, are present in the seminal fluids of OX5034 remains unanswered." (0397 p. 6) [Footnote Omitted]

Two commenters suggested that the transgenic proteins expressed in OX5034 may be present in the saliva of biting females.

The Center for Food Safety stated:

> "While Oxitec previously claimed there are no proteins unique to the GE mosquito in the saliva of the OX513A mosquito, no data is [sic] presented for OX5034. Even if Oxitec provided data for a few hundred or thousand OX5034, it is still possible that some unknown percent of OX5034 mosquitoes do have these proteins in their saliva." (0396 p. 8)

Friends of the Earth stated that:

> Also of concern is that biting female GE mosquitoes may inject a novel engineered protein (*tTAVOX5034 and DsRed2-OX5034)* 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, nor has it determined the protein's allergic or toxic potential. Oxitec's claim about the potential toxicity or allergenicity from biting GE mosquitos and the lack of exposure to biting females depends on the assumption that females do not survive to adulthood. In reality, survival may occur if resistance develops or because of environmental exposure to tetracycline (see more detail in the section above and our previous submission)." (0394 pp. 4-5) [Footnotes omitted] [Emphasis in Original]

One commenter (0397) stated that inhalation exposure to OX5034 transgenes may occur through dust formation within homes. GMO Free Florida stated that EPA's assumption that access by OX5034 to indoor spaces is expected to be minimal is contradicted by peer reviewed studies which routinely find male *Ae. aegypti* indoors in homes:

> *"Aedes aegypti* males are routinely found in homes. Males are attracted to humans and produce pheromones which attract other males and females. This poses an allergy risk, including inhalant allergies, which has not been evaluated… Many studies have indicated that male *Aedes aegypti* are attracted to humans and routinely land on humans. . . , even in the absence of females, male *Aedes aegypti* are themselves attracted to humans. *"We used semi-field experiments to demonstrate robust attraction of male Ae. aegypti to humans. Human-baited traps captured up to 25% of released males within 15 min, whereas control traps without humans as bait failed to capture males. Rapid attraction to humans was further demonstrated through videography. Males swarmed around and landed on human subjects, with no activity recorded in paired unbaited controls. The absence of female Ae. aegypti in these experiments rules out a hypothesis by Basrur et al. (2020) that males are attracted not to the human, but to host-seeking females near humans.*" (0397 pp. 1-2) [Footnotes Omitted] [Emphasis in Original]

Given their contention that *Ae. aegypti* males routinely enter homes, GMO Free Florida furthermore argued that releases of male OX5034 will result in even more males in-home:

> "In the case of the OX5034 releases, where potentially hundreds of millions of *Aedes aegypti* may be released, the number of males will likely significantly increase indoors. It is expected that the release of *Aedes aegypti* males will increase the presence of male *Aedes aegypti* in homes because as more males are present, more males will follow females indoors. Male *Aedes aegypti* also produce pheromones that attract other males and females. Therefore, the release of millions of male *Aedes aegypti* will increase the number of males and females around humans." (0397 pp. 1-2) [Footnotes Omitted]

Arguing that field releases will result in more *Ae. aegypti* mosquitoes indoors, GMO Free Florida stated that:

> "Peer reviewed studies routinely find male *Aedes aegypti* indoors in homes. In fact, in studies where male *Aedes aegypti* were released a greater number of males were often found in homes compared to the number of females in homes and a greater number of mosquitoes, both male and female, were found indoors compared to outdoors. Therefore, the release of male *Aedes aegypti* will increase the number of males and females around humans." (0397 p. 9) [Footnote Omitted]

GMO Free Florida also argued that EPA's evaluation must consider the possibility of large numbers of *Ae. aegypti* males entering and remaining in-home:

> " . . . , the long duration of this experiment of 24 months, and short life expectancy of *Aedes aegypti* males, this is likely to cause male *Aedes aegypti* to die, and hence remain indoors, and contribute to dust formation. If an additional 24 months is added to this experiment, as is proposed, this will only further increase the amount of *Aedes aegypti* males that will enter and die in homes." (0397 pp. 1-2)

GMO Free Florida (0397) furthermore stated that *Ae. aegypti* has been found to deposit eggs in indoor spaces, noting that:

> " . . . peer reviewed evidence indicates that *Aedes aegypti* do deposit eggs indoors in homes. In fact, peer reviewed evidence, *"found that the infestation of indoor containers by Ae. aegypti was greater than outdoor containers"* and concluded, *"Many studies have demonstrated that Ae. aegypti rest indoors, feed indoors and oviposit indoors."* (0397 pp. 1-2) [Footnotes Omitted] [Emphasis in Original]

The commenter went on to argue that, in light of references [see 14 in 0397[10]] indicating eggs will be oviposited in-home:

> "While male adults may be the only adults that carry OX5034 traits, the larvae also express the novel proteins tTAV-OX5034 and DsRed2-OX5034, and . . . such larvae will be present indoors in homes." (0397 p. 2)

Stating that these "potentially allergenic novel proteins expressed by males may be inhalant allergens" and "according to SECTION G PROPOSED EXPERIMENTAL PROGRAM, 5265 million male mosquitoes are released for the program," GMO Free Florida argued that:

> "It is well established in the peer reviewed literature that *Aedes aegypti* derived allergens, via emanations and detritus, are present in the air and allergy to insects has a significant bearing on the clinical characteristics of allergic bronchial asthma patients. Therefore these initial releases, and increase in overall *Aedes aegypti* numbers, would very likely increase the number or severity of Type I allergic respiratory disorders." (0397 pp. 1-2) [Footnotes Omitted] [Emphasis in Original]

[10] L. J. Hribar, J. J. Vlach, D. J. Demay, S. S. James, J. S. Fahey and E. M. Fussell (2004) Mosquito Larvae (Culicidae) and Other Dipteria Associated With Containers, Storm Drains, and Sewage Treatment Plants in the Florida Keys, Monroe County, Florida"
http://web.archive.org/web/20101206000141/http://www.fcla.edu/FlaEnt/fe87p199.pdf

C. Hazard Considerations

Comments (0396, 0397, 0445) were received revolving around hazard considerations. Two commenters (0396, 0397) expressed concern that the tTAV-OX5034 and DsRed2-OX5034 proteins engineered into OX5034 could be toxic or allergenic.

The Center for Food Safety and GMO Free Florida stated that:

> "Although Oxitec claims, *"tTA and its variants, such as tTAV, have been used in fungi, rodents, plants, and mammalian cultures with no known non-target adverse effects on the environment or human health"* signs of toxicity and neurotoxicity have been reported in mice expressing the tTA protein." (0396 p. 9; 0397 p. 27) [Footnotes Omitted] [Emphasis in Original]

GMO Free Florida also stated that:

> "DsRed2 has cytotoxic effects on bacteria as well as human cells and this may increase in the presence of doxycycline." (0397 p. 27) [Footnotes Omitted]

One commenter (0397) questioned whether the two transgenic proteins expressed in OX5034 could be allergens.

GMO Free Florida argued that:

> "Not only may the initial increased number of *Aedes aegypti* lead to increases in allergic responses, but the novel proteins expressed by the OX5034 may also lead to allergic responses." (0397 p. 2)

And:

> "It has already been established that proteins from the OX513A share sequences identical to known human allergens and this is likely true for OX5034 as well. These proteins could be present in the saliva of OX5034 females or as inhalant allergens from OX5034 males." (0397 p. 8)

GMO Free Florida furthermore described the results of a database search which were conducted as "a search for OX513A through SDAP - Structural Database of Allergenic Proteins, and using FAO/WHO Allergenicity Rules based on Sequence Homology, and exact match for 6 contiguous amino acids," the matches identified correspond to:

> "Sequence matches were identified which correspond to human allergen sequence matches, including inhalant allergens, from castor beans, eastern honey bee, white birch tree and potatoes. Castor bean inhalant allergens may result in anaphylactic shock, and the inhalant Bet v

> 1 allergen from white birch, which shares sequences with the OX513A, affects over 100 million allergic patients. While sharing matching sequences alone doesn't guarantee an allergic reaction, further studies are required when there are exact matches for 6 contiguous amino acids, and it appears no studies have been done. When Oxitec submitted documentation to the EPA for OX5034, however, they did not publicly provide the sequences for either protein making it impossible for physicians and scientists to search the allergen databases." (0397 p. 6) [Footnotes Omitted]

With regard to the question of whether receipt of seminal fluid from OX5034 males could affect wild females seeking and feeding behavior or saliva protein composition, GMO Free Florida and the Center for Food Safety stated that:

> "Oxitec must also conduct studies to determine if the saliva of wild female *Aedes aegypti* in the Keys and California is altered once they are inseminated by GE *Aedes aegypti* males as it is unknown if this may alter the saliva of the wild female and perhaps even cause the wild female to have unique proteins in their saliva." (0396 p. 8; 0397 p. 7)

One commenter raised chromothripsis as a hazard consideration.

Robert Gregory (0445) stated that:

> "My very serious question and comment concerns chromothripsis. Have you considered the short and long term effects of what you are doing including the phenomenon of chromothripsis? . . . ." (0445 p. 1)

EPA Response to Comments in Unit V.A - Comments on Potential for Disease Transmission from OX5034 Mosquitoes. Regarding the statement that mosquitoes that carry the OX5034 trait could contain factors that alter female behavior through seminal fluids transferred from OX5034 males to wild-type females, the commenter did not provide sufficient information in the context of this EUP amendment and extension to enable EPA to further respond, i.e., why the seminal fluid would be expected to be different and why those differences would lead to changes in disease transmission and, more specifically, an increase in disease transmission. EPA notes that longevity, fecundity, as well as the vectorial capacity of OX5034 were evaluated as part of the original 2020 risk assessment for the OX5034 EUP (EPA-HQ-OPP-2019-0274-0359). Both fecundity and longevity of OX5034 were found to be within the expected range of what has been documented in the scientific literature for *Ae. aegypti* (Original risk assessment: Unit II.C.3.a., Unit II.6.c., and Unit II.6.d., and Memorandum on vectorial capacity: EPA-HQ-OPP-2019-0274-0351).

Regarding the comments on the potential effects of arbovirus prevalence through OX5034 releases, EPA finds the outcomes hypothesized by the commenter highly unlikely in the context of this EUP amendment and extension due, in part, to the low efficacy of horizontal transmission (i.e., between

adult mosquitoes) and vertical transmission (i.e., female to offspring) of arboviruses between *Ae. aegypti* individuals. Horizontal arbovirus transmission from a homozygous OX5034 male to a wild female is unlikely because, as stated in the risk assessment for the original 2020 OX5034 EUP, "the conditions under which OX5034 is reared are unlikely to result in the presence of arboviruses in the colony" (EPA-HQ-OPP-2019-0274-0359). For an already infected wild female to be able to infect other females, two horizontal transmission events will have to occur, with each of these events individually having a low probability, i.e., transfer from the infected female to a male and from that male to another female. This is because horizontal transmission is itself not an efficient process even under controlled laboratory conditions. Representative numbers in *Ae. aegypti* range from 11% for CHIKV, 31.6% for DENV-2, and 50% for ZIKA (Mavale *et al*., 2010[11]; Silva Campos *et al*., 2017[12]; Sánchez-Vargas, *et al*., 2018[13]). Should a male still be infected through mating in the wild, that male's chances of passing the infection to another female is even lower than would be expected from the efficacy of the horizontal gene transfer itself. This is because (1) the OX5034 treatment area is overflooded with OX5034 males and (2) the expected decrease in female population over the duration of the trial. Both factors reduce the chances for that male to mate. Together, EPA finds the scenario described by the commenter highly unlikely.

Like horizontal transmission, vertical transmission is an inefficient process and only a few, if any, of the offspring will acquire the infection from an infected female. For example, under optimal laboratory conditions designed to determine whether *Ae. aegypti* can vertically transmit a specific viral strain, recorded filial infection rates ranged within the order of 1:84 - 1:290 for ZIKV[14,15] or as low as 1:1,300 for DENV-4.[16] Nevertheless, assuming that a small number of offspring does acquire the infection and survives into adulthood, because females in the test area are more likely to mate with OX5034 males (due to a high OX5034 males to wild-type males ratio), any infected offspring is more likely to be male (because OX5034 female offspring dies) and males do not expose humans to viruses. Lastly, it is worth

[11] Mavale, Mangala & Parashar, Deepti & Sudeep, Anakkathil & Gokhale, Mangesh & Ghodke, Youwaraj & Geevarghese, Geevarghese & Arankalle, Vidya & Mishra, Akhilesh. (2010). Venereal Transmission of Chikungunya Virus by Aedes aegypti Mosquitoes (Diptera: Culicidae). The American journal of tropical medicine and hygiene. 83. 1242-4. 10.4269/ajtmh.2010.09-0577.

[12] Campos, Stéphanie & Fernandes, Rosilainy & Santos, Alexandre & Miranda, Rafaella & Telleria, Erich & Ferreira, Anielly & Castro, Marcia & Failloux, Anna-Bella & Bonaldo, Myrna & Lourenço-de-Oliveira, Ricardo. (2017). Zika virus can be venereally transmitted between Aedes aegypti mosquitoes. Parasites & Vectors. 10. 10.1186/s13071-017-2543-4.

[13] Sánchez-Vargas, I., Harrington, L. C., Doty, J. B., Black, W. C., 4th, & Olson, K. E. (2018). Demonstration of efficient vertical and venereal transmission of dengue virus type-2 in a genetically diverse laboratory strain of Aedes aegypti. *PLoS neglected tropical diseases*, *12*(8), e0006754. https://doi.org/10.1371/journal.pntd.0006754

[14] Ciota, A. T., Bialosuknia, S. M., Ehrbar, D. J., & Kramer, L. D. (2017). Vertical Transmission of Zika Virus by Aedes aegypti and Ae. albopictus Mosquitoes. *Emerging infectious diseases*, *23*(5), 880–882. https://doi.org/10.3201/eid2305.162041

[15] Thangamani, S., Huang, J., Hart, C.E., Guzman, H. and Tesh, R.B., 2016. Vertical transmission of Zika virus in Aedes aegypti mosquitoes. *The American journal of tropical medicine and hygiene*, *95*(5), p.1169.

[16] Rosen, L., Shroyer, D.A., Tesh, R.B., Freier, J.E. and Lien, J.C., 1983. Transovarial transmission of dengue viruses by mosquitoes: Aedes albopictus and Aedes aegypti. *The American journal of tropical medicine and hygiene*, *32*(5), pp.1108-1119.

noting that any of these scenarios of arbovirus transmission are only possible if a virus that is vectored by *Ae. aegypti* is present in the test area.

Regarding the comment that OX5034 females or females that mated with OX5034 males may have a different saliva composition, it is important to note that only non-biting male mosquitoes will be released into the environment, which do not pose an infection hazard. Regarding the potential for changes in the saliva composition of mated females, those females are themselves not genetically engineered and the commenter did not provide information on the mechanism by which the seminal fluid of OX5034 could have the proposed effects. Thus, EPA is unable to respond further.

Regarding the comments on the effects of the mosquito biome on vectorial capacity, EPA has previously responded to this consideration in Unit VIII.C. "Comments on Potential for Environmental Releases of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations" in the original 2020 Response to Comments document on page 77 (EPA-HQ-OPP-2019-0274-0355). Because no new arguments were brought forward, the Agency refers the commenter to the previous responses. However, it is relevant to note that the EPA response that was quoted by commenter 0397 goes on to read in relevant part: *"(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-HQ-OPP-2019-0274-0355)." These points further address the commenter's concern.

Regarding the comments requesting that the vectorial capacity of OX5034, including its capability to vertically transmit arboviruses, be tested, EPA carefully considered information provided on the vectorial capacity, longevity, and fecundity associated with OX5034 in the memorandum cited by one of the commenters (EPA-HQ-OPP-2019-0274-0351 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*.").

Regarding the comment on the potential effects from increasing the number of male *Ae. aegypti* in the test areas on disease transmission, the commenter proposes that the release of OX5034 males would result in more Zika infected females by increasing the likelihood for a single infected wild-type female to mate. However, this conclusion is based on the assumption that a proportion of wild-type female mosquitoes relevant to disease transmission outcome would not have mated in the absence of OX5034 releases, but no evidence to support this assumption was provided.

Regarding the comment that the increase in male density in the release areas may lead to a spread of diseases, EPA finds it unlikely that mosquito densities will be reached that are high enough for the proposed scenario to occur. However, if mosquito densities were so high as to disrupt mating, any incomplete sperm transfer from males to females would be reasonably expected to result in incomplete arbovirus transfer. Given that arbovirus transfer through mating is already an inefficient process, mating disruption would only be expected to further lower the possibility of disease transmission (see response to Unit V.A.1).

Regarding the comment that relates the spread of disease to the proximity of male mosquitoes around humans and the ability of males to attract other mosquitoes, the commenter did not provide information as to why the presence of additional males (i.e., OX5034 males) will necessarily result in increased number of females around humans through the proposed mechanisms. EPA notes that there is a general expectation that the total number of females in the release area will decline over time as a result of the OX5034 releases, leaving overall fewer females to bite humans. Further, while male pheromones play a role in mating, it is not clear from the comment to what degree the additional OX5034 males would help guide females to humans and if any of those females would have otherwise failed to locate their host. It is generally accepted that females are not dependent on cues from male mosquitoes to locate their host. Rather, females are able to do so primarily by sensing carbon dioxide that is exhaled with the human breath, but also certain compounds found in body odors.[17,18] Relying on these cues allows female mosquitoes to independently secure a blood meal to support the development of their eggs.

Regarding the comment that a reduction in the *Ae. aegypti* population may result in an "increased spread of disease," the commenter did not substantiate their hypothesis that a reduction in competition (presumably between females for human hosts) would result in an increase in multiple host-feeding behavior, which could subsequently lead to an increase in disease spread. Thus, EPA is unable to comment further. However, regarding the general notion that a reduction in the number of females could result in an increase in disease transmission, while the cited article[19] states that "Ae. aegypti *mosquitoes persist and effectively transmit dengue virus even at very low population densities because they preferentially and frequently bite humans*," contrary to the commenters hypothesis, the same article also states that "*The currently proposed indices for* Ae. aegypti *density at best weakly correlate with human dengue infection, and their relationship to disease is understudied*." In other words, based on the article, there is no expectation that a reduction in *Ae. aegypti* population would lead to an increase in dengue transmission. In fact, once a certain minimum threshold required to sustain disease transmission is reached, any further reduction in female abundance is expected to ultimately reduce disease transmission and occurrence.

[17] Cardé, R.T., 2015. Multi-cue integration: how female mosquitoes locate a human host. *Current Biology*, *25*(18), pp.R793-R795.
[18] Raji, J.I. and DeGennaro, M., 2017. Genetic analysis of mosquito detection of humans. *Current opinion in insect science*, *20*, pp.34-38.
[19] Scott, T.W., Takken, W., Knols, B.G. and Boëte, C., 2002. The ecology of genetically modified mosquitoes. *Science*, *298*(5591), pp.117-119

Regarding the comments that testing of OX513A coincided with increases in disease resistance, commenters did not provide information that allows for the evaluation of the relevance of these statements for OX5034, which is a different mosquito product developed by Oxitec and which is the only product covered under this EUP amendment and extension. For more information on the causes of Zika and the transmission of the Zika virus please see the following website from the Centers for Disease Control and Prevention at www.cdc.gov/zika/index.html.

With regard to the comment discussing the potential for viral evolution in response to OX5034 mosquitoes, as noted by the Agency in EPA's response to this comment in the Response to Comments Document for the original OX5034 EUP (Docket number: EPA-HQ-OPP-2019-0274-0355), the publication[20] cited by commenter 0394 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.

EPA Response to Comments in Unit V.B - Exposure Considerations. Regarding the comments on the potential for oral exposure to OX5034, as outlined in the human health evaluation of the original 2020 EUP for OX5034 (Docket number: EPA-HQ-OPP-2019-0274-0355; Unit II.B.3.b.ii), the likelihood of oral exposure to the OX5034 mosquitoes was determined to be negligible. There are several factors that limit the likelihood of oral exposure and the chances of accidental ingestion in addition to the fact that OX5034 is not to be used in food. Examples are the modes of mosquito application, such as the larval releases in largely enclosed containers that are expected to result in a staggered release (i.e., lower densities) of adult male mosquitoes at the release site and the preference of *Ae. aegypti* to breed in non-potable water sources such as leaf axils and tires.

Regarding the comments on the potential for dermal exposure to OX5034, as outlined in the human health evaluation of the original 2020 EUP for OX5034 (Docket number: EPA-HQ-OPP-2019-0274-0359; Unit II.B.3.b.i), the likelihood of dermal exposure to the OX5034 mosquitoes (males and females) was determined to be negligible. Female OX5034 mosquitoes do not have a potential for dermal exposure as they are not expected to be present in the environment (Docket number: EPA-HQ-OPP-2019-0274-0359; Unit II.B.3.a). With regard to the possibility of the transfer of the transgenic proteins from OX5034 males to wild-type females, EPA finds that even if such a transfer from males to females were to occur and the female were to subsequently land on bare skin, exposure to these substances is expected to be negligible. This is because the tTAV-OX5034 and DsRed2-OX5034 proteins are present within the insect's cells, as noted in the risk assessment for the original 2020 EUP, cited above. Another way in which female mosquitoes interact with humans is through biting. However, the possibility that wild-type female mosquitoes may acquire genetically engineered proteins through mating with OX5034 males and transfer these proteins to people through biting, is an implausible scenario. For GE proteins to be found in the saliva of wild females, a number of steps would have to occur, each of which in and of themselves

[20] Medlock, J., Luz, Paula M., Struchiner, Claudio J., and Galvani, Alison P. (2009) The Impact of Transgenic Mosquitoes on Dengue Virulence to Humans and Mosquitoes. The American Naturalist 174, 565-577.

are highly unlikely. For example, for the aforementioned scenario to occur, the genetically engineered proteins must first be present in the seminal fluid of the OX5034 male mosquito and present at high concentrations. Those proteins would then have to cross multiple physiological barriers in the female without being diluted too much or degraded (i.e., leave female reproductive tract, survive and migrate through the female mosquito body, enter female salivary gland, be expelled through saliva), which is implausible.

Regarding the comment on the potential inhalation exposure to *Ae. aegypti* in homes, EPA acknowledged the possibility that some male mosquitoes may be present in homes in its risk assessment of the original 2020 OX5034 EUP and concluded that they are not expected to contribute significantly to indoor dust formation within the context of the trials (Docket number: EPA-HQ-OPP-2019-0274-0359; Unit II.B.3.b.iv). *Ae. aegypti* is generally not considered an indoor home pest in the US. Some of the reasons are biological in nature. For example, *Ae. aegypti* mate in aerial swarms. This behavior requires clustering of a number of mosquitoes, with the larger abundance of individuals found outdoors. Further, males rely on plant nectar as their source of nutrition, and these sources again are most abundant outdoors. In addition to these biological considerations, the expectation is that mosquito access into homes will be significantly restricted in the test areas. Releases of OX5034 will occur in geographical locations in which *Ae. aegypti* (as well as other mosquito species) is already present, which means houses in that area are more likely to be outfitted with screens on windows and doors, providing a physical barrier to entry. Further, *Ae. aegypti* is present primarily in warmer areas of the US and many households in these locations rely on air conditioning for climate control. Use of air conditioning reduces the need for other ventilation or cooling mechanisms such as opening of windows and doors, which again minimizes mosquito access into homes. Lastly, not all of the individuals that gain access to homes necessarily contribute to dust formation as they may try to exit the home in search of food and mating opportunities outdoors. Thus, as stated in the risk assessment for the original 2020 EUP, inhalation exposure from dust formation for tTAV-OX5034 and dsRed2-OX5034 is considered negligible in the context of this EUP amendment and extension. Regarding the comment on the oviposition behavior of *Ae. aegypti*, for the reasons outlined above, EPA finds it unlikely that indoor dwellings provide a likely oviposition site for *Ae. aegypti* in the trial areas and thus inhalation exposure from these sources is not expected to be relevant in the context of this EUP amendment and extension.

EPA Response to Comments in Unit V.C. - Hazard Considerations. Regarding the comments on the hazard considerations, as noted in the risk assessment of the original 2020 EUP for OX5034 (Docket number: EPA-HQ-OPP-2019-0274-0359), the risk that a pesticide poses to human health is a function of hazard and exposure. As discussed in Unit II.B.3.b of that document, the most relevant exposure to tTAV-OX5034 and DsRed2-OX5034 would be expected from biting OX5034 females if they were present in the environment. However, 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 in the context of the original 2020 EUP as well as the current EUP amendment and extension. EPA carefully assessed the penetrance of the OX5034 female-lethal trait

as part of the original EUP request for OX5034. The results of that review are discussed in Unit II.B.3.a of the original risk assessment.

Regarding the comment that OX5034 females or females that mated with OX5034 males may have a different saliva composition, it is important to note that only non-biting male mosquitoes will be released into the environment which do not pose an allergy hazard. Regarding the potential for changes in the saliva composition of mated females, those females are themselves not genetically engineered and the commenters did not provide information on the mechanism by which the seminal fluid of OX5034 could have the proposed effects. Thus, EPA is unable to respond further.

With regard to the comment on chromothripsis, the commenter did not provide sufficient information to allow EPA to determine how chromothripsis might be relevant to testing of OX5034 mosquito.

VI. Comments on Environmental Considerations, including Non-Target Organisms and Threatened and Endangered Species

A number of comments were received on environmental considerations (0344, 0369, 0380, 0383, 0388, 0394, 0396, 0397, 0399, 0401, 0402, 0413, 0432, 0434, 0436, 0439, 0442, 0444, 0448, 0449, 0460, 0461). Comments on environmental considerations revolve around the potential for releases of OX5034 mosquitoes to have effects on other organisms, including endangered or threatened species. Comments suggested effects to non-target organisms could be direct such as through consumption of OX5034 mosquitoes or indirect through dynamic changes in the ecosystem. Comments also discussed the potential for releases of OX5034 mosquitoes to impact the population dynamics of other mosquito species. Finally, comments suggested other ways that releases of OX5034 mosquitoes may affect the environment, including the potential for the spread of antibiotic resistant microbes, introgression of OX5034 strain genetic material or the tTAV-OX5034 and/or DsRed2-OX5034 transgenes into wild *Ae. aegypti* populations, horizontal transfer of the transgenes into other animals or soil bacteria and bioaccumulation of the transgenic proteins or of heavy metals.

A. Comments on the Potential Effects on Non-Target Organisms from Consumption of OX5034 Mosquitoes

Regarding effects through consumption, some comments (0383, 0397) challenged EPA's risk assessment finding that non-target organisms would not consume OX5034 as a significant part of their diet. Others expressed concern for non-target organisms that may consume OX5034 mosquitoes.

1. Potential and degree of exposure to OX5034 mosquitoes by non-target animals

Comments discussed the degree to which non-target organisms may be exposed to OX5034 mosquitoes through the consumption of OX5034 eggs or larvae.

Contesting EPA's conclusion that the role *Ae. aegypti* play in the predator diet is limited due to the preferred larval habitat of Ae. aegypti mosquitoes, GMO Free Florida stated that:

> " . . . this ignores that dragonfly larvae, for example, may consume large amounts of *Aedes aegypti* larvae and tadpoles can also consume large amounts of *Aedes aegypti* eggs and larvae. For example, the green tree frog native to Florida consumes mosquito larvae. Salamander larvae also consume large amounts of mosquito larvae at a level comparable to the mosquitofish. Dragonfly larvae, salamander larvae and tadpoles are often found in the same larval habitat as *Aedes aegypti*, in fact peer reviewed evidence indicates *Aedes aegypti* prefer to lay their eggs in water sources with tadpoles of the genera *Polypedates, Bufo, Ramanella, Euphlyctis and Hoplobatrachus* in them.*"* (0397 p. 21 & 28) [Footnotes Omitted]

Specifically contesting the EPA claim that, *"it does not appear that the salamanders . . . feed significantly on Ae. aegypti larvae"*, GMO Free Florida contended that:

> " . . . the EPA provides no evidence for such a claim. In fact, the EPA notes, *"the Tiger Salamander, Ambystoma tigrinum, was found to readily consume mosquito (Culicidae, species not identified) larvae based on 26% of analyzed stomach samples containing remnants of larvae."* If the species was not identified in this study, the EPA has no basis to claim the tiger salamander does not feed significantly on Aedes aegypti, considering according to Brodman and Dorton, *"mosquitoes were third most abundant prey taxon and were found in 26% of the tiger salamander larvae ranking second behind cladocerans".* This would certainly indicate that tiger salamanders feed significantly on mosquito larvae. In the study by Brodman and Dorton it states, *"mosquito larvae were a preferred prey"* and, *"We observed that tiger salamander larvae can eat a large number of mosquito larvae in short periods of time."* (0397 p. 21-22 & 28-29) [Footnote Omitted][Emphasis in the Original]

GMO Free Florida reasoned that:

> "Since salamander larvae and tadpoles are confined to those larval habitats, unable to walk or hop out, they will consume large amounts of *Aedes aegypti* as a primary food source. This is especially important since this is a large consumption of OX5034 early in their development. As the EPA mentions, *"as many of the aquatic insects that may consume OX5034 larvae are larvae themselves and thus more susceptible to even low-level toxins, additional certainty regarding the lack of toxicity to aquatic insect larvae could be gained through a larval feeding study prior to a Section 3 registration."* (0397 p.21) [Emphasis in the Original]

GMO Free Florida continued to challenge EPA's risk assessment conclusion that the role *Ae. aegypti* play in the predator diet is limited due to the preferred larval habitat of Ae. aegypti mosquitoes, stating that:

> "Nine species of dragonflies and three species of damselflies found in the Keys can eat mosquitoes, carnivorous plants like small butterwort, lizards like the green anole and amphibians like green tree frog tadpoles, etc. all can eat mosquitoes. . . . dragonfly larvae, for example, may consume large amounts of Aedes aegypti larvae and tadpoles can also consume large amounts of Aedes aegypti eggs and larvae. For example, the green tree frog native to Florida consumes mosquito larvae. Salamander larvae also consume large amounts of mosquito larvae at a level comparable to the mosquitofish." (0397 p. 21) [Footnotes Omitted]

GMO Free Florida also stated that:

> "Mammals in the Florida Keys such as the bat *Molossus molossus* eat mosquitoes as well as other insects, like dragonflies, that consume mosquitoes". (0397 p. 25) [Footnotes Omitted]

GMO Free Florida also stated that in addition to direct consumption, organisms may be exposed via bioaccumulation of the transgenic proteins:

> "Even organisms that have not consumed OX5034 larvae, pupae, or adults may still be consuming these transgenic proteins. Transgenic proteins can adsorb to the surface of algae, cyanobacteria and macrophytes that are then consumed by higher organisms up the food web, which then bioaccumulate and are then consumed by even higher organisms up the food web. In this way nearly every endangered species may be consuming these transgenic proteins unique to OX5034 . . . ." (0397 p. 22 & 25) [Footnote Omitted]

GeneWatch UK stated that:

> "Ingestion may also be a potential exposure route for . . . animals, as females are expected to die at the larval stage in the water where they breed. Further, GE males are expected to be released in large numbers and to survive in each generation and could be swallowed by . . . animals." (0383 p. 5)

2. <u>Potential for effects on non-target animals through consumption of OX5034 mosquitoes</u>

Some comments (0394, 0396, 0402) expressed concern over the potential for non-target organisms to consume OX5034 mosquitoes and stated that additional testing is needed.

Friends of the Earth stated that:

> "The risks of ingestion, whether intentional or unintentional, of GE mosquitoes by mammals, reptiles, birds, or other organisms, have not been adequately assessed." (0394 p. 4)

The Center for Food Safety stated that:

> "Nine species of dragonflies and three species of damselflies found in the Keys can eat mosquitoes, the bat *Molossus molossus*, carnivorous plants like small butterwort, lizards like the green anole and amphibians like green tree frog tadpoles, etc. all can eat mosquitoes. Dragonfly larvae, for example, may consume large amounts of *Aedes aegypti* larvae. . . . So, what happens if the transgene is consumed by any or all of these species?" (0396 p. 7) [Footnote Omitted]

The Center for Food Safety stated that:

> "If GE mosquito larvae are deposited in pet dishes, a dog, cat, etc. may drink the water and consume some larvae in the process. . . . So, what happens if the transgene is consumed by any or all of these species?" (0396 p. 7 & 25)

Friends of the Earth stated that:

> "It is unclear what the impacts of the GE mosquitoes on wild animals, including endangered or threatened species, and farm animals are. There is no publicly available data about any feeding trials for mammals or birds, which given the prevalence of endangered species in California, is critical. There is also missing information about mosquito predators or prey, which could be impacted by GE mosquito releases and fluctuating mosquito populations. More feeding trials are needed to assess the risk of ingestion to wild species that eat mosquitoes. Ingestion may also be a potential exposure route, as females are expected to die at the larval stage in the water where they breed." (0394 p. 2)

Lisa Kelly and her family commented that "much further research is done prior to any release of GMO mosquitoes" because:

> "Not enough is known about how GMO mosquitoes will affect Bats and Bat populations. Bats are known to consume mosquitoes as part of their natural diet. If mosquitoes are genetically modified, how will this affect Bat populations? Will consuming GMO mosquitoes be harmful to Bats? Bats the world over are already in serious decline. Humans need to proceed with utmost caution and make certain that these GMO mosquitoes aren't going to contaminate mosquitoes as a food source." (0402 p. 1)

B. Comment on the Potential Effects on Native Pollinators

One commenter (0397) expressed concern over the potential effect that OX5034 mosquito releases may have on native pollinator resources.

GMO Free Florida argued that the release of millions of OX5034 males may adversely impact native pollinators:

> "Native pollinators in the Florida Keys and California often rely on nectar as a food source. The addition of millions of OX5034 which also consume nectar is adding environmental resistance in the form of increased competition for food, and this could affect the biotic potential of pollinator populations." (0397 p. 30)

C. Comments on the Potential to Affect Mosquito Population Dynamics

Comments (0383, 0394, 0397, 0439, 0461) discussed whether dynamic interactions could occur between *Ae. aegypti* and *Ae. albopictus* populations as well as between *Ae. aegypti* and other species.

GeneWatch UK stated that:

> "The risk assessment argues that no species rely on *Aedes aegypi* [sic] if the population falls (p.43-49), but it does not address the impacts on human health or biodiversity of fluctuations (high numbers during releases, followed by potential reductions and then perhaps a rebound in population numbers), or movement of wild mosquitoes from one area to another in response to the releases (as appears to have happened in the Cayman Islands and elsewhere in response to earlier releases of the OX513A strain)." (0383 p. 6)

GeneWatch UK further stated that:

> ". . . , there is no analysis of potential response of the competitor species *Aedes albopictus* (which is also an important disease vector) to fluctuations in *Aedes aegypi* [sic], despite concerns that suppression of *Aedes aegypti* (if successful, even temporarily) could lead to more *Aedes albopictus* moving into an area, due to reduced competition. This issue is discussed in more detail in our previous submission. As Oxitec's former Chief Scientific Officer, Luke Alphey has 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*...'. Both species are present in California (as has been previously noted for Florida), yet no information is provided in the application regarding any surveys of mosquito species in the proposed release areas." (0383 p. 7) [Footnotes Omitted]

Friends of the Earth stated that:

> "Oxitec's GE mosquitos could also increase other vectors for diseases like dengue fever. If Oxitec's mosquitoes 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 Asian Tiger Mosquito is one of the most

> invasive mosquito species, and research has shown it is a possible vector for dengue fever and other tropical diseases, possibly leading to more harm to human health. The Asian Tiger Mosquito is widespread in the USA, including in Florida." (0394 p. 4) [Footnotes Omitted]

GMO Free Florida added "*Aedes albopictus* has replaced the *Aedes aegypti* in some urban environments in the past such as in Mobile, Alabama . . . " making "it likely that *Aedes albopictus* will move into the test areas in the Keys or California where *Aedes albopictus* is not currently present" and that:

> *"Aedes albopictus* also transmits mosquito borne diseases such as dengue fever and chikungunya as well as Zika. *Aedes albopictus* infected with dengue, as well as West Nile Virus, eastern equine encephalomyelitis, Cache Valley and La Crosse virus have been found in North America." (0397 p.12) [Footnotes Omitted]

Contending that once the trial is over, *Ae. aegypti* and *Ae. albopictus* could co-occur, GMO Free Florida argued that:

> "This is extremely relevant since, for example, data strongly suggests that *Aedes albopictus* acted as the major vector of both dengue and chikungunya in Libreville in 2007, impacting on the epidemiology of both viruses in this area, even though both *Aedes aegypti* and *Aedes albopictus* were present. Therefore, outbreaks can occur which are caused by *Aedes albopictus* which do not involve *Aedes aegypti* and vice versa. So this release could create a scenario where 2 separate species can independently cause an outbreak of such diseases instead of only 1 species which currently exists. The EPA responded to comments about *Aedes albopictus* displacing the *Aedes aegypti* in the test areas, but the EPA did not respond to the concerns about co-occurrence of *Aedes aegypti* and *Aedes albopictus* in the test area because of the experiment with OX5034." (0397 p. 13) [Footnotes Omitted]

GMO Free Florida further stated that:

> *"Aedes albopictus* may move in as *Aedes aegypti* population decreases causing a co-occurrence of both species creating a scenario where two vectors of diseases will be present in areas where only one was present before the trial. This co-occurrence of two species would also cause an increase in the number and severity of allergic reactions as people in test areas would then be exposed to an additional species that has allergens which they are not desensitized to." (0397 p. 12)

GMO Free Florida added that:

> "Although at least 8 allergens have been found in *Aedes aegypti* saliva, more than 16 allergens have been found in *Aedes albopictus* saliva. If *Aedes albopictus* are able to establish in the trial area because of a reduction in *Aedes aegypti* this could increase the number of allergic

> reactions. Since most people are bitten by mosquitoes in or around their home, Keys and California residents are not likely to have been largely exposed to *Aedes albopictus* and therefore natural desensitization likely does not exist among Keys and California residents in the test areas. Therefore, due to Keys and California residents low or absent natural immunity, having little or no previous exposure to *Aedes albopictus*, they are at an increased risk of severe reactions to mosquito bites and this is especially true for young children. OX5034 mosquitoes present a unique risk since current control methods in the Keys and California, such as the use of the larvicide Vectobac - Bacillus thuringiensis israelensis, often target both Aedes aegypti and *Aedes albopictus*. However, OX5034 mosquitoes would target only *Aedes aegypti* possibly causing a reduction in only *Aedes aegypti* which could create an opportunity for *Aedes albopictus* to enter the Keys and California and establish itself due to reduced competition with *Aedes aegypti*, whereas current control methods do not carry the same risk." (0397 p. 13) [Footnotes Omitted]

Anonymous (0439) added that:

> "In addition, even if the experiment were to result in a decrease in the Aedes aegypti mosquito, it is likely that other varieties of mosquitoes, such as the Aedes albopictus (Asian tiger) mosquitoes, which also transmit dengue and several other viruses (including chikungunya), will increase in numbers to fill the new niche. In other words, other species of wild mosquitoes in the environment, including in peoples' homes and backyards." (0439 p. 1) [Footnote Omitted]

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

Comments (0397, 0401, 0413, 0439, 0449, 0460, 0461) on the use of antibiotics in production on OX5034 mosquitoes revolve around concerns that use of the antibiotic could lead to an increase in antibiotic resistance in disease causing microorganisms. One commenter was concerned about the potential spread of the tetracycline antibiotic itself (0449). Finally, another commenter contested EPA's risk assessment conclusion that the "*question of whether disposal of wastewater could spread antibiotic resistance does not apply"* (0397).

GMO Free Florida stated that:

> "Tetracycline use can increase the risk of tetracycline resistant bacteria, . . ." (0397 p. 13)

Anonymous (0439) stated that:

> "The use of tetracycline to breed the GE mosquitoes in the laboratory also risks spreading antibiotic resistance, which could pose a major risk to human and animal health." (0439 p. 1) [Footnote Omitted]

Californians for Pesticide Reform and Institute for Responsible Technology stated that:

> "Tetracycline and related antibiotics are used in rearing the GE mosquitoes, increasing the risk of creating antibiotic-resistant diseases. A petition signed by nearly three dozen physicians in the Florida Keys demanded tests to rule out this danger prior to any release. It was ignored." (0401 p. 3; 0461 p. 5)

Comment 0460 representing Mass Mailer and John Ulloth stated that:

> "Oxitec's mosquitoes are raised in tetracycline and may spread antibiotic-resistant bacteria, such as staph and MRSA." (0413 p. 2; 0460 p. 2)

GMO Free Florida stated that:

> "Tetracycline resistant bacteria could also be found on GE *Aedes aegypti* due to the potential for tetracycline resistant bacteria in the laboratory where tetracycline is used and GE *Aedes aegypti* are potentially exposed." (0397 p. 14)

GMO Free Florida identified "a causal pathway for genetically engineered *Aedes aegypti*'s gut bacteria acquiring antibiotic resistance genes as they are fed on antibiotics in the laboratory":

> " . . . although, *'Moll et al. (2001) describe an effective mechanism to eliminate gut microorganisms during mosquito metamorphosis and adult emergence. Bacteria found in recently emerged non-fed adults are present from the larval and pupal stage and, therefore, have some adaptations to overcome this mechanism.'* Other researchers have concluded that based on their observations Serratia odorifera was transstadially transmitted in *Aedes aegypti* from larvae to adult, ... Other studies provide similar results. A recent study suggests that *Aedes aegypti* larvae cannot survive past the first instar without gut microbiota. In this study they used ampicillin to kill the gut bacteria in second and third instar *Aedes aegypti* and they did not molt. However, if the *Aedes aegypti* larvae had ampicillin resistant bacteria they survived to adulthood. If we assume this research is correct and microbiota are needed for *Aedes aegypti* to survive, we can also assume OX5034 mosquitoes are like conventional mosquitoes and also require microbiota to survive. Since OX5034 female mosquitoes are exposed to tetracyclines this would likely kill all of the microbiota except the tetracycline resistant bacteria." (0397 p. 14) [Footnotes Omitted]

GMO Free Florida stated that:

> "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'*. Therefore, Oxitec must conduct studies to determine if tetracycline resistant microbiota are found in GE mosquitoes." (0397 p. 14) [Footnotes Omitted]

Concerned that tetracycline itself could be spread in the environment, Joni Stellar (0449) stated that:

> "These pesky little insects are the basis of a vast food web including amphibians, birds, fish, mammals and other insects. The effects of adding tetracycline via mosquitos to a wide swath of species is unknown and frightening." (0449 p. 1)

Contesting EPA's statement in its risk assessment that "*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,"* GMO Free Florida stated that:

> "This does not take into consideration that the waste will be discarded somewhere, whether in the U.S. or not, and this waste has the potential to increase antibiotic resistant pathogens. Tetracycline is an antibiotic used for humans and may therefore cause human harm if pathogens become resistant to it. This antibiotic is used to treat MRSA and its non-medical use may lead to tetracycline resistant MRSA. Tetracycline is also used in the treatment of canine heartworm which is transmitted by *Aedes aegypti*. Therefore, tetracycline resistant bacteria harbored on Oxitec's mosquitoes could render tetracycline useless for canine heartworm treatment." (0397 p. 15) [Footnotes Omitted]

E. Comments Concerning Other Means by Which OX5034 Might Affect the Environment

Comments in this category (0383, 0394, 0397, 0432, 0442, 0460, 0461) discuss the potential for the release of OX5034 mosquitoes to result in the unintended spread or accumulation of various biological substances (e.g., genes, proteins) in the environment. Specifically, commenters suggest the potential for the spread of OX5034 background strain genetics, spread of OX5034 transgenes to other organisms, and bioaccumulation of heavy metals.

1. Potential for introgression of OX5034 genes into the local *Ae. aegypti* mosquito populations

Several comments (0383, 0394, 0397, 0460, 0461) argued that OX5034 genes would introgress into local wild populations and supported their conclusions by referencing information from the releases of Oxitec's OX513A *Ae. aegypti* mosquito in Brazil. Two commenters hypothesized about potential consequences of such an occurrence.

Comment 0460 representing Mass Mailer stated that:

> "Despite Oxitec's claims to the contrary, GMO mosquito genes were found to have spread to the wild population 3 years after a similar experiment in Brazil." (0460 p. 2)

The Institute for Responsible Technology stated that:

> "Three years after Oxitec's Brazilian release, an independent follow-up study [*Link provided to Evans et al., 2019*] identified a large number of 'hybrid' mosquitoes, whose genomes carried DNA from both the GE insects and the local natural variety. It was unknown if these insects—new to nature—would be harder to kill with insecticides, more likely to transmit disease in their bites or have a survival advantage to increase their numbers over time. However, what was certain is that Oxitec was wrong, and they inadvertently changed nature's gene pool with unpredictable consequences." (0461 p. 1)

GMO Free Florida added that:

> "Even in the absence of the transgenes, the OX5034 is still a foreign strain of *Aedes aegypti* which is not currently present in the Florida Keys or California. According to Oxitec, for the OX5034 a laboratory strain was mixed with a Latin American wild strain which has a background comprising genetics from ten different colonies of *Aedes aegypti* originating in Mexico. This has led to concerns that this hodge podge, lab strain mixed with Mexican strain mixed with the strain present in the Florida Keys, California or elsewhere, could lead to hybrid vigor. Hybrid vigor can lead to a more robust population of mosquito than those which are not a mixture of different strains." (0397 p. 10) [Footnote Omitted]

Friends of the Earth stated that:

> "The concerns about introgression of the Aedes aegypti into wild type mosquitoes and the potential vectoral capacity have not been addressed, despite evidence from Brazil highlighting that the genetic material from Oxitec's GE mosquitoes were found in wild mosquitoes. The vectoral capacity of Oxitec's GE mosquitoes should be fully assessed, as well as the potential vectoral capacity of hybrid mosquitoes that could carry the genetic material from Oxitec's GE mosquito. This information should be made publicly available ahead of a public comment period." (0394 p. 3) [Footnotes Omitted]

GeneWatch UK referring to the "Summary of the Data and Information Related to Vectorial Capacity Presented for the New Product OX5034 (EPA File Symbol: 93167-EUP-E). . . , dated February 12, 2020" stated that:

> "This may be the document on vectorial capacity that has now been published as EPA-HQ-OPP-2019-0274-0351. However, this is just a "summary' and any full consideration of this issue (if it has been undertaken) has still not been published for public scrutiny. It is worth noting that this

> document recognises the importance of this issue, stating, "*the degree of introgression is likely to be significantly higher than that of the OX513A strain due to higher larval survival rates (approx. 5% in OX513A versus 50% in OX5034) […]Traits associated with a disease vectoring species such as Ae. aegypti that may carry risk if introgressed into a wild population are likely to be linked to vectorial capacity, including vector competence, fecundity, and longevity*" (EPA-HQ-OPP-2019-0274-0351). Although this document concludes that, "*it is not expected that introgression of OX5034 strain genetics would increase the vector competence of the wild mosquito population*", no evidence is provided in the document to support such a conclusion. Further, the EPA's subsequent response to comments goes much further than this document when it states (p. 31) that the risk is "*negligible*". It is hard to see how this risk can have been properly assessed without testing the vectorial capacity of the introduced strain and any potential admixed strains." (0383 p. 5) [Footnote Omitted] [Emphasis in Original]

2. <u>Potential for the OX5034 transgenes, or fragments of the transgenes, to transfer to an animal's microbiome and/or soil bacteria</u>

Some comments (0397, 0432, 0442) questioned the claim that recombinant DNA detectable by PCR was not found in animals and birds that consumed food containing a transgene. One comment (0432) thought marsh ecosystems have not been adequately studied for the potential for genetic transmission and potential horizontal gene transfer. Two commenters (0397, 0442) questioned whether the *piggyBac* system could be a potential mechanism for horizontal gene transfer.

GMO Free Florida contested Oxitec's claims that, *"several studies have addressed the fate of ingested DNA in mammals and birds, . . . fed with glyphosate tolerant soybean"* [or] *"with recombinant Bacillus thuringiensis corn. In none of those studies was recombinant DNA detectable by PCR in various samples"*, stating that:

> " . . . , studies suggest that transgenes, from the consumption of GE foods, may transfer to the intestinal microbiota of humans. Animal studies where animals have consumed GE foods suggest that some transgenes in GE foods may be able to transform oral bacteria. That some transgenes in GE foods may survive passage through the small intestine and have been detected in feces. The possibility that consumption of GE mosquitoes by insectivores may result in the transgene(s) being found in feces leads to potential risks such as soil microbes being exposed to the feces, and thus the transgene(s), and the potential for gene transfer from the transgene from feces to soil microorganisms. Even fragmented, or partially degraded, transgenes may be able to transform oral bacteria or soil bacteria. This is important as fragmented transgenes from OX5034 are likely to remain in the environment for a long enough period for transformation to occur. Animal studies, not included in Oxitec's assessment, have observed transgenes in blood, kidneys, liver, heart, muscle, brain and milk of animals fed GE foods as well as their offspring." (0397 p. 27-28) [Footnotes Omitted]

Andy Peri stated that:

> "The complexity of marsh ecosystems the potential for genetic transmission and potential horizontal gene transfer have not been adequately studied." (0432 p. 1)

Two commenters suggested the *piggyBac* system as a potential mechanism for horizontal gene transfer.

GMO Free Florida stated:

> "Oxitec's claims, *"The piggyBac transposable element . . . is prevented from moving within or outside the genome of its host because it does not encode or produce the associated transposase enzyme that is necessary for such movement*." However, Joe Cummins and Mae-Wan Ho state, *" . . . transposase function can be supplied by a 'helper' transposon. Such helper transposons are ubiquitous." . . . . , "the disabled vector carrying the transgene, even when stripped down to the bare minimum of the border repeats, was nevertheless able to replicate and spread, basically because the transposase function enabling the piggyBac inserts to move can be supplied by 'helper' transposons. Such helper transposons are potentially present in all genomes...Although each transposon has its own specific transposase enzyme that recognizes its terminal repeats, the enzyme can also interact with the terminal repeats of other transposons, and evidence suggest "extensive cross-talk among related but distinct transposon families" within a single eukaryotic genome*. *Oxitec must reassess and include this new information."* (0397 p. 32-33) [Footnotes omitted] [Emphasis in Original]

S. Rigali raised concerns that the *piggyBac* transposon could transfer to human cells or into the wild mosquito population, stating:

> "The use of the piggyBac transposon has been plagued by problems of instability in transformed Aedes aegypti; and large unstable tandem inserts of the piggyBac transposon were prevalent. In spite of instability and resulting genotoxicity, the piggyBac transposon has been used extensively also in human gene therapy. Several human cell lines have been transformed, even primary human T cells using piggyBac. These findings leave us little doubt that the transposon-borne transgenes in the transgenic mosquito can transfer horizontally to human cells. The piggyBac transposon was found to induce genome wide insertion mutations disrupting many gene functions." (0442 p. 1) [Footnotes Omitted]

The commenter further noted that:

> "In particular, it would be precautionary to know if any of the genetic material inserted into the Aedes aegypti mosquitos using the piggyBac transposon survives in wild populations in and around the area in which the Oxitec mosquitos were released in 2011 and 2012. . . . If indeed

> such genetic material does persist in the wild this fact would need to be taken into account by regulators as a significant risk factor in their consideration of any future releases." (0442 p. 1)

3. Potential for heavy metals to bioaccumulate in and affect the food chain

Some comments (0397) questioned whether heavy metals could bioaccumulate in and affect the food chain, including affecting animals that consume mosquito larvae and eggs.

GMO Free Florida stated that "[E]vidence indicates that *Aedes aegypti* can bioaccumulate heavy metals over several generations," and that:

> "While heavy metal bioaccumulation over generations may not be a great concern for wild *Aedes aegypti*, where *Aedes aegypti* breed in different containers and consume different feeds over several generations, this is a concern for laboratory reared *Aedes aegypti* that will be exposed to the same environment and likely consume the same feed, be reared in the same habitats, etc. over generations. This is especially important because OX5034 mothers are fed on horse blood, and horse blood can contain heavy metals." (0397 p. 30) [Footnotes Omitted]

GMO Free Florida stated that evidence indicates that:

> ". . . consumption of the *Aedes aegypti* with high heavy metal content adversely impacts dragonflies that consume them. Heavy metal exposure is also detrimental to tadpoles." (0397 p. 28) [Footnote Omitted]

GMO Free Florida argued that a number of species consume mosquito larvae and eggs, including "tadpoles of the genera *Polypedates, Bufo, Ramanella, Euphlyctis and Hoplobatrachus" "*salamander" and "green tree frog" and that:

> "Heavy metals may even bioaccumulate and harm predators that consume the organisms that consume the OX5034. This poses a risk for organisms higher up in the food web that eat the organisms that consume the OX5034 larvae, pupae or adults and not just the organisms that directly consume the OX5034. This becomes an even greater risk for organisms such as frogs or salamanders who would consume OX5034 early in their life cycle and then as adults consume organisms, such as dragonflies, that also consumed OX5034. However, even organisms that have not consumed OX5034 larvae, pupae, or adults may still be consuming these heavy metals." (0397 p. 29)

GMO Free Florida further argued that:

> "Heavy metals can adsorb to the surface of algae, cyanobacteria and macrophytes that are then consumed by higher organisms up the food web, which then bioaccumulate and are then

> consumed by even higher organisms up the food web. In this way nearly every endangered species may be consuming these heavy metals from OX5034." (0397 p. 29) [Footnote Omitted]

GMO Free Florida then suggested threatened or endangered species that "are also very likely to consume large amounts of *Aedes aegypti"* and perhaps be exposed to heavy metals by OX5034, including:

> "Amphibians: *'Ambystoma californiense, Ascaphus truei, Batrachoseps sp, Batrachoseps campi, Batrachoseps pacificus pacificus, Batrachoseps relictus, Batrachoseps simatus, Batrachoseps stebbinsi, Bufo canorus, Bufo exsul, Bufo microscaphus californicus, Ensatina eschscholtzii croceator, Ensatina eschscholtzii klauberi, Hydromantes sp, Hydromantes brunus, Hydromantes platycephalus, Hydromantes shastae, Plethodon elongatus, Plethodon stormi, Rana aurora aurora, Rana aurora draytoni, Rana boylii, Rana cascadae, Rana muscosa, Rana pretiosa, Rana yavapaiensis, Rhyacotriton variegatus, Scaphiopus hammondii*."'
>
> Reptiles: *'Anniella pulchra nigra, Anniella pulchra pulchra, Clemmys marmorata marmorata, marmorata, Cnemidophorus hyperythrus, Cnemidophorus tigris multiscutatus, Coleonyx switaki, Coleonyx variegatus abbotti, Elgaria panamintina, Eumeces skiltonianus interparietalis, Heloderma suspectum cinctum, Sceloporus graciosus graciosus, Sceloporus graciosus vandenburgianus, Uma notata notata, Xantusia henshawi gracilis, Xantusia vigilis sierrae*.'
>
> Insects: '*Capnia lacustra, Ammopelmatus kelsoensis, Ammopelmatus muwu, Macrobaenetes kelsoensis, Ambrysus funebris, Pelocoris shoshone, Agabus rumppi, Chaetarthria leechi, Hydroporus hirsutus, Hydroporus leechi, Hydroporus simplex, Hygrotus curvipes, Hygrotus fontinalis.'*
>
> Birds: *'Agelaius tricolor, Aimophila ruficeps canescens, Amphispiza belli belli, Chlidonias niger, Geothlypis trichas sinuosa, Histrionicus histrionicus, Ixobrychus exilis hesperis, Laterallus jamaicensis, Plegadis chihi, Toxostoma lecontei macmillanorum.'"* (0397 p. 29-30)

EPA Response to Comments in Units VI.A - Comments on the Potential Effects on Non-Target Organisms from Consumption of OX5034 Mosquitoes. With regard to comments noting the potential for non-target organisms, including threatened and endangered species, to be exposed to OX5034 mosquitoes via consumption, the Human Health and Environmental Risk Assessments associated with the original EUP request, and this amendment, concluded that due to species-specific behavioral traits of *Ae. aegypti* resulting in its preferential habitat being largely limited to areas surrounding human dwellings and its preferential breeding sites being largely composed of man-made containers, the potential of exposure of non-target organisms to OX5034 *Ae. aegypti* males is limited. Due to the OX5034 mosquitoes releases occurring in residential sites and to biological traits of *Ae. aegypti* (e.g., anthropophilic, limited dispersal), it is reasonable to find that exposure to OX5034 *Ae. aegypti* mosquitoes by listed species is expected to be limited.

Commenters noted that non-target organisms have the potential to consume mosquitoes (non-species specific) and may consume *Ae. aegypti* mosquitoes; however, no comments were provided demonstrating that *Ae. aegypti* is a significant or critical food source for any non-target organism. As no new arguments were brought forward regarding the degree of exposure or the potential for indirect effects from OX5034 mosquito releases (i.e., reduction of a potential food source), the Agency refers the commenters to the Human Health and Environmental Risk Assessments associated with the original EUP request (see Unit II.D.2 of the original risk assessment EPA-HQ-OPP-2019-0274-0359) and this amendment (see Unit III.C.3.b of the current risk assessment).

With regard to the comments expressing concern that non-target animals such as birds, dragonflies, bats, frogs, salamanders, or lizards will be adversely affected by consumption of OX5034 mosquitoes or larvae containing the OX5034 trait, EPA previously evaluated whether there was any risk to non-target organisms, which includes listed species and the organisms specifically mentioned in the comments [i.e., on birds, dragonflies, bats, frogs, salamanders, or lizards] from the consumption of the OX5034 *Ae. aegypti* mosquito. The Human Health and Environmental Risk Assessments associated with the original EUP request and this amendment concluded that no adverse effects from consumption are expected – that is, there is a reasonable expectation of no discernible effects for nontarget organisms – based on the mode of toxicity of tTAV, bioinformatics analyses, and acute oral toxicity studies. Relevant findings are available in the Human Health and Environmental Risk Assessment associated with the original EUP in Unit II.D.2, "Ecological exposure and risk characterization," and the Human Health and Environmental Risk Assessment associated with this amendment in Unit III.C.3.a, "Direct effects," located in the docket established for this action (EPA-HQ-OPP-2019-0274).

With regard to the comment stating that non-target organisms that do not typically eat mosquitoes may be exposed to and adversely affected by the OX5034 transgenic proteins via bioaccumulation in the food chain, the citation provided by the commenter examines systemic insecticidal transgenic toxins in plants (e.g., *Cry* proteins), which display known toxicity to specific non-target organisms. However, as described in this section, the transgenic proteins in the OX5034 mosquito are not expected to pose adverse effects from consumption by any non-target organism based on the mode of toxicity of tTAV, bioinformatics analyses, and acute oral toxicity studies. Therefore, following the same rationale underlying the conclusion of no adverse effects for any non-target organism from the direct consumption of OX5034 mosquitoes, no adverse effects are expected from the hypothetical scenario raised by the commenter. Although EPA finds that no adverse effects are expected should bioaccumulation of the transgenic proteins occur, EPA also notes that the commenter did not provide rationale as to why bioaccumulation would be expected for the OX5034 transgenic proteins.

EPA Response to Comments in Unit VI.B - Comment on the Potential Effects on Native Pollinators.
With regards to the comment that OX5034 may outcompete native pollinators for nectar resources, EPA concludes that this is unlikely for a number of reasons: 1) As discussed in the Human Health and Environmental Risk Assessment associated with this amendment in Unit III.C.3.b, "Indirect effects," due

to *Ae. aegypti*, and therefore OX5034 mosquitoes, being anthropophilic and thus found primarily near human dwellings, the types of nectar resources utilized by *Ae. aegypti* mosquitoes is likely to be limited to those found in residential settings, 2) pollinators have access to alternative food sources, 3) OX5034 males will not persist indefinitely to consume nectar resources, and 4) the use of OX5034 mosquitoes is anticipated to suppress the local wild *Ae. aegypti* population thereby resulting in the overall reduced use of nectar resources by *Ae. aegypti* mosquitoes.

EPA Response to Comments in Unit VI.C - Comments on the Potential to Affect Mosquito Population Dynamics. Regarding comments stating that fluctuations in *Ae. aegypti* populations due to OX5034 releases may affect mosquito population dynamics, please refer to the Response to Comments associated with the original 2020 EUP under section, "EPA Responses to Comments in Unit VIII.B.2. – Population Dynamics; Possibility that Other Mosquito Species Might Displace Ae. aegypti" (EPA-HQ-OPP-2019-0274-0355). Because no new arguments were brought forward, the Agency refers the commenters to these previous responses.

EPA notes that one commenter referenced a gene drive, but as explained in the Response to Comments associated with the original 2020 EUP under section, "EPA Responses to Comments in Unit IV – Comments on Characteristics of OX5034" (EPA-HQ-OPP-2019-0274-0355), the OX5034 *Ae. aegypti* mosquito does not contain a gene drive, rather OX5034 is a self-limiting, locally acting, mosquito designed to suppress local *Ae. aegypti* populations.

EPA Response to Comments in Unit VI.D - Comments on Potential for Environmental Releases of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations. Regarding comments expressing concern over antimicrobial resistance, EPA notes that many of the comments are not relevant to the OX5034 male mosquitoes for release because these mosquitoes are not reared using tetracycline. While tetracycline containing growth trays were used for a previous Oxitec mosquito, the OX513A mosquito, tetracycline will not be used in the US facilities or rearing boxes of the OX5034 mosquito. Please see section "EPA Response to Unit VIII.C. – Comments on Potential for Environmental Releases of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations" of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274-0355). Because no new arguments were brought forward, the Agency refers the commenters to these previous responses.

Regarding the comment concerning the possible spread of tetracycline itself from the release of OX5034 mosquitoes, because no tetracycline is used in the development of the male mosquitoes for release, tetracycline will not be introduced to the environment via those mosquitoes.

Regarding comments that tetracycline disposal from rearing facilities in Europe may contribute to worldwide antibiotic resistance, EPA notes that facilities using tetracycline for colony rearing are not located in the United States and thus are not within EPA's jurisdictional bounds and are therefore outside the scope of this assessment.

EPA Response to Unit VI.E - Comments Concerning Other Means by Which OX5034 Might Present Risk to the Environment. Regarding the comments on introgression, EPA received similar comments from the public on the original EUP for the OX5034 strain proposed for testing in Florida in 2020. EPA has responded to these comments in Unit V.A. on page 31 (regarding the findings published in Evans *et al*., 2019) and Unit XI. on page 105 (regarding insecticide resistance) of the document entitled "Response to Comments to the Notice of Receipt of an Application for an Experimental Use Permit Number 93167-EUP-E." That document (-0355) can be found in the Docket # EPA-HQ-OPP-2019-0274 on www.regulations.gov. Because no new arguments were brought forward, the Agency refers the commenters to these previous responses. One of the commenters contended that the Agency did not provide evidence in the document to support the conclusion that "*it is not expected that introgression of OX5034 strain genetics would increase the vector competence of the wild mosquito population*." EPA emphasizes that the basis of the Agency's conclusion is reported, in part, in the first half of the sentence which was omitted from the quote and which reads "*In summary, 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, […].*" Additional explanation is provided in the same document (EPA-HQ-OPP-2019-0274-0351) in Unit III. "Conclusions" and data and information that supported this conclusion are also presented in Unit II of that document (-0351), "Summary." One of the commenters referred to a study by Evans and colleagues from 2019, which examined Oxitec's first generation product, OX513A. That study was evaluated by EPA and the Centers for Disease Control and Prevention as part of the original assessment for the OX5034 EUP, and the results of that evaluation are published in the same memorandum discussed in this paragraph. As the commenter has not brought forward new arguments, the Agency refers the commenter to that memorandum (EPA-HQ-OPP-2019-0274-0351) as well as EPA's previous responses in the Response to Comments document for the original EUP in Unit V.A (EPA-HQ-OPP-2019-0274-0355).

With regard to comments that OX5034 transgenes may transfer to an animal's microbiome or soil bacteria, EPA finds this scenario to be unlikely as horizontal gene transfer is a relatively rare event, and in order for a gene to spread within a microbial population, it would have to be not only stably integrated into the genome but also confer some type of selective advantage to the bacterium. The transgenes present in OX5034 carry no such selective advantage, and in fact, would be expected to be quickly selected against due to the lethality of the t-TAV system in its host organism. Because horizontal gene transfer is relatively rare and because the transgenes in OX5034 mosquito carry no selective advantage, EPA concludes that the scenario outlined by the commenter is very unlikely.
Regarding the comment that the *piggyBac* system could be a potential mechanism for horizontal gene transfer, as discussed in the original 2020 risk assessment, transgenic *Ae. aegypti* created using *piggyBac*-derived transposon systems have been reported to be genetically stable, possibly due to a low proportion of transposon-specific piRNAs in this mosquito species (Sethuraman et al. 2007).[21] In agreement with this observation is the finding that remobilization of the OX5034 expression cassette in

[21] Sethuraman, N., M. J. Fraser, P. Eggleston, and D. A. O'Brochta. 2007. Post-integration stability of piggy Bac in Aedes aegypti. Insect Biochemistry and Molecular Biology **37**:941-951.

OX5034 *Ae. aegypti* has not been observed in over 27 generation equivalents. The original risk assessment furthermore concluded that "re-excision from the genome is not expected to occur as the transposase is only transiently expressed in transformed cells and the integrated cassette does not itself encode for a transposase enzyme." The scientific article referenced by the commenter reported on one *piggyBac*-based construct that showed a degree of instability when transformed into *Ae. aegypti* (Adelman *et al*., 2004)[22]. One of the characteristics of this observed instability was the absence of the expected Mendelian inheritance pattern in subsequent mosquito generations. In contrast to this observation, OX5034 transgenes were shown to be inherited in a Mendelian fashion for several generations. Note that the other citation provided by the commenter (Adelman et al 2002)[23] is a symposium abstract by the same authors that appears to discuss the same findings published in 2004.

Regarding the comment on whether the genetic material inserted using the *piggyBac*-derived transposon (i.e., the genetic construct containing the transgenes) would persist in wild *Ae. aegypti* populations, as noted in Unit II.C.2. of the original risk assessment (EPA-HQ-2019-0274-0359), it is expected that the OX5034 transgenes would disappear from the environment within 10 mosquito generations. The review further expands on this assessment, stating that "it is likely that in the field when OX5034 males compete against local *Ae. aegypti* males, the trait will decline faster than found in the modeling and caged population studies, as additional fitness costs associated with the OX5034 trait have been recorded, like reduced egg clutch size in matings with OX5034 homozygous males." Thus, persistence of the OX5034 transgenes is not expected to occur.

With regard to comments discussing the potential for the horse blood fed to the OX5034 colony to contain heavy metals and for those heavy metals to be passed on to OX5034 male offspring and potentially lead to the bioaccumulation of heavy metals in the environment, the commenter did not provide sufficient information as to why the horse blood used in OX5034 colony rearing is expected to contain heavy metals at levels different from what may be consumed by wild *Ae. aegypti* mosquitoes. Therefore, EPA finds that it is unlikely that the heavy metals in the bloodmeal of parental mosquitos as passed on to their male offspring would significantly contribute to the risk from heavy metal environmental pollution.

VII. Efficacy of OX5034 To Suppress Mosquito Populations

Comments (0367, 0374, 0375, 0383, 0372, 0373, 0384, 0392, 0393, 0394, 0397, 0400, 0408, 0412, 0399, 0413, 0414, 0439, 0451, 0453, 0459) were received on the question of whether OX5034 would be

[22] Adelman ZN, Jasinskiene N, Vally KJ, Peek C, Travanty EA, Olson KE, Brown SE, Stephens JL, Knudson DL, Coates CJ, James AA. Formation and loss of large, unstable tandem arrays of the piggyBac transposable element in the yellow fever mosquito, Aedes aegypti. Transgenic Res 2004, 13(5), 411-25.

[23] Adelman ZN1, Jasinskiene N1, Peek C1, Travanty EA2, Olson KE2, James AA1. Instability of the piggyBac element in transformed Aedes aegypti. ISMIS 2002. Abstracts of the Fourth International Symposium on Molecular Insect Science. 70pp. Journal of Insect Science, 2, 17.

effective in suppressing mosquito populations in the field. Some comments (0367, 0374, 0375, 0392, 0393, 0459) expressed the opinion that OX5034 would be efficacious. Some comments (0373, 0383, 0384, 0394, 0397, 0399, 0400, 0401, 0408, 0412, 0413, 0414, 0439, 0451, 0453, 0457) questioned whether OX5034 would be efficacious. Some of these comments suggested OX5034 efficacy should be evaluated against other means of mosquito control.

A. Comments Questioning Whether OX5034 Would Be Efficacious

Several commenters argued that OX5034 was not efficacious, basing their concern on arguments that OX513A had not been proved efficacious. Some of these comments equated efficacy to reduction in disease frequency. Others based their arguments on reports analyzing or commenting on data generated during testing of OX513A as well as comments from officials of countries where testing of OX513A had been conducted.

1. Comments equating efficacy to reduction in disease frequency

Several comments (0383, 0372, 0394, 0412, 0413, 0439, 0451) equated efficacy to reduction of disease transmission.

GeneWatch UK, questioning whether OX5034 would be efficacious, argued that "Oxitec's proposed experimental releases will not show whether its GE mosquitoes really make a difference to disease, or even if they will reduce the numbers of the target wild mosquito (*Aedes aegypti*)," and stated that:

> " . . . no assessment is planned of the impact on risk of disease. The EPA states, "…*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…*" (p.106, EPA-HQ-OPP-2019-0274-0355). However, there is a complex relationship between mosquito numbers and disease transmission and evidence of population suppression is not sufficient to show a reduction in disease, or risk of disease." (0383 p. 2) [Emphasis in Original]

Friends of the Earth stated that:

> "Although Oxitec claims that the GE mosquito could reduce 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 also not provided data to assess whether population reductions of Aedes aegypti, if they did occur, would lead to disease eradication or reduction." (0394 p. 2)

Anonymous (0439) stated that:

> "To date, genetically engineered mosquitoes have been an expensive failure. Studies have shown that they are not 100% sterile, there is no evidence of their effectiveness at disease reduction, and their release may result in the spread of more mosquito borne diseases. To date, none of the field trials in the Cayman Islands, Malaysia, or Panama effectively reduced the Aedes aegypti mosquito population or disease rates." (0439 p. 1) [Footnote Omitted]

Anonymous (0439) further stated that:

> "There is [sic] no data to support Oxitec's claims that open releases of GE mosquitoes will reduce incidence of mosquito borne diseases. The Environmental Protection Agency (EPA) notes that Oxitec's trials are not set up to test for disease reduction. There have been field trials in the Cayman Islands, Panama, Malaysia, and Brazil. Releases in Panama and Malaysia have stopped, and the Cayman Islands ended its plans for larger releases because of concerns that the GE mosquitos would not be effective in reducing dengue rates." (0439 p. 1) [Footnotes Omitted]

Karen Barranco and John Ulloth stated that:

> "Oxitec . . . has not proven that their GMO mosquitoes reduce disease transmission." (0412 p. 1; 0413 p. 2)

The Institute for Responsible Technology stated that:

> " . . . the company has yet to provide any conclusive evidence that their GE mosquitoes have ever reduced the incidence of mosquito-borne Zika, Dengue, and Chikungunya diseases." (0461 p. 4)

2. <u>Comments arguing that OX5034 may not be efficacious based on reports or comments from officials of countries where testing had been conducted on OX513A</u>

Some comments (0370, 0383, 0399, 0453) based their arguments that OX5034 would not be efficacious on information from countries where OX513A was tested.

GeneWatch UK further questioned whether efficacy is being evaluated, stating that:

> " . . . Freedom of Information requests to the Cayman Islands' Mosquito Research and Control Unit revealed comments from scientists with access to the data such as:
>
> • "*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*."
>
> • "*To date all the measures recorded have shown no significant reduction in the abundance of Aedes aegypti in the release area*."

> "In reality, Oxitec's proposed experimental releases will not show whether its GE mosquitoes really make a difference to disease, or even if they will reduce the numbers of the target wild mosquito (*Aedes aegypti*)." (0383 p. 2) [Footnote Omitted] [Emphasis in Original]

Belinda Mostert stated that:

> "Enough data from previous locations aka the Cayman Islands, Brazil and African countries proves this to be a failed experiment." (0453 p. 1)

B. Comments Arguing for Alternative Approaches

Some comments (0388, 0394, 0397, 0401, 0414, 0419, 0420, 0430, 0434) argued that alternative approaches to *Ae. aegypti* mosquito control are preferable to the use of OX5034. These commenters argued for the use of (1) Wolbachia infected mosquitoes, and (2) other control methods.

1. Arguments as to why methods other than use of OX5034 would be preferrable

Two comments (0394, 0397) explained why they thought other methods of controlling *Ae. aegypti* were preferrable to OX5034.

Friends of the Earth, arguing that eliminating one disease vector opens a niche for others such as *Ae. albopictus*, stated 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." (0394 p. 4)

GMO Free Florida stated that:

> "The current control methods do not include purposely increasing the mating of *Aedes aegypti*, leading to an increase in population if the lethality trait fails, which makes Oxitec's proposal riskier, with regards to disease spread, than current control methods." (0397 p. 32)

2. Comments preferring use of Wolbachia-infected mosquitoes for mosquito control

Some comments (0399, 0400, 0414, 0401) preferred the use of *Wolbachia*-infected mosquitoes for *Ae. aegypti* control.

Californians for Pesticide Reform stated that:

> "Safer alternatives already exist, such as through use of the benign bacteria Wolbachia." (0401 p. 1)

Meagan Morrison Hull stated that:

> "Wolbachia is a far more successful and less controversial mosquito abatement technology, more compatible with biological systems." (0399 p. 3)

Edward Russo stated that:

> "If the EPA would simply do an alternatives analysis, . . .you would stumble over Wolbachia." (0414 p. 1)

Florida Keys Environmental Coalition stated that:

> "Wolbachia enabled Cytoplasmic Incompatibility (CI) that can more effectively address chemically resistant Aedes Aegypti with low risk of environmental harm as well as address any potential back filling Aedes Albopictus, an assurance that Oxitec's product cannot provide." (0400 p. 3)

Institute for Responsible Technology and Californians for Pesticide Reform stated that:

> "In parallel with the development of GE mosquitoes, other companies developed and verified effective methods for reducing mosquito populations or rendering mosquitoes incapable of transmitting diseases using a benign bacteria called Wolbachia. The bacteria are already found throughout the insect world and are an example of a tool that does not introduce the unpredictable side effects from genetic engineering, as well as the threat from export markets, the risks of horizontal gene transfer, the potential dangers from a GE mosquito bite, and more." (0401 p. 4; 0461 p. 7) [Emphasis in Original]

GMO Free Florida stated that:

> "Other strategies to control the *Aedes aegypti* also rely on releasing male mosquitoes, however, strategies such as the use of the bacterium *Wolbachia* do not carry this risk of spreading diseases as *Wolbachia* inhibits viral replication in infected *Aedes aegypti* stopping the mosquito from spreading diseases such as Zika virus, dengue fever, yellow fever, chikungunya as well as the parasites that cause avian malaria and the filarial nematodes responsible for canine and feline heartworm. Unlike OX5034, Wolbachia-infected mosquitoes could reduce the *Aedes aegypti* population without the risk of increasing mosquito-borne diseases. In fact, a study done in Australia where *Wolbachia* infected mosquitoes were released show a 96% reduction in dengue fever." (0397 p. 9) [Footnotes Omitted]

GMO Free Florida stated that with other mosquito control techniques, even if "male *Aedes aegypti* are released . . . there are built in redundant safety measures with those techniques" that OX5034 do not have because if "the OX5034 mates with wild females harboring diseases, the male can then acquire diseases such as Zika from the infected female through venereal transmission" and argued that:

> "If experiments used Wolbachia or IIT/SIT then this wouldn't happen. There wouldn't be 50% of offspring surviving to spread diseases and/or the mosquitoes would be largely resistant to diseases like dengue, Zika, chikungunya, yellow fever, filarial nematodes, plasmodium, west nile virus, etc. anyway. This makes Oxitec's proposal riskier, with regards to disease spread, than current control methods and similar experiments." (0397 p. 30-31) [Footnotes Omitted]

3. Comments on other control measures

Some comments (0373, 0384, 0408) argued for other control measures or simply indicated that other measures should be prioritized.

Jo Rand stated:

> Why can't you buy dragonfly's? [sic] . . . Dragon flys [sic] eat millions of mozzys!!! (0373 p. 1)

Mary Hollowell stated that:

> "The solutions to malaria-causing mosquitoes are wearing natural insect repellant, wearing long pants and sleeves, and avoiding infested areas. Tinkering with Mother Nature, however, is unacceptable." (0384 p. 1)

Maggie Mistal stated that:

> "They also still spray for mosquitos and drop pellets and do all the same mosquito control approaches as done previously." (0408 p. 1)

C. Comments Arguing That OX5034 is Likely Efficacious

Six commenters (0367, 0374, 0375, 0392, 0393, 0459) argued that OX5034 is likely to be efficacious.

Mustapha Debboun stated that:

> "I am very encouraged by the effective and promising results from Oxitec's release of Friendly Ae. aegypti in South America, particularly in Brazil and encourage the EPA to approve Oxitec's

> EUP amendment to expand the use of this important and needed technology in California." (0367 p. 1)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District, stated that:

> "FKMCD has been investigating and studying Oxitec's Genetically Modified mosquitoes for more than ten years. Since April 2021 Oxitec's Second-Generation Technology of its Friendly Mosquitoes have been emerging from small "boxes" in numerous sites in our County based on our EUP with the EPA. Thus far, everything has gone as anticipated and data is being gathered for the EPA to evaluate at the conclusion of the trials. The only issue we have experienced is the requirement from the Florida Department of Agriculture and Consumer Services (FDACS) to pause the trial when a named tropical storm or hurricane is headed our way. This has caused us to interrupt the program two times in the first half of this hurricane season. This is one reason we need the two-year extension on our EUP should we experience another storm this year and need more time to complete the trial." (0374 p. 1)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District, added that:

> "After nearly five months into the EUP, we see the Oxitec Friendly Mosquitoes (non-biting males only) are emerging from the small "boxes" and mating as anticipated. We are also realizing that the deployment of eggs followed by adult mosquitoes emerging from the small "boxes" is much easier, much less labor intensive and overall more long term sustainable than rearing and releasing adult mosquitoes as was required in Oxitec's First Generation technology and in other competing technologies." (0374 p. 1)

Phil Goodman, Commissioner and Chairman of the Board of the Florida Keys Mosquito Control District, summarized that:

> "If the Oxitec Program proves successful for us as it has in other countries, is approved for commercial use by the EPA, and we use it, we will definitely not eliminate pesticide spraying for nuisance mosquitoes, but we should be able to reduce our total pesticide spraying significantly. Some environmental groups initially expressed concerns which were addressed by the EPA. No one then really considered that reduced spraying was possible. Now, everyone should be pleased to learn that reduced chemical spraying is possible if the Oxitec Friendly Mosquito can significantly reduce the local Aedes aegypti population." (0374 p. 2)

Norman C. Leppla, University of Florida, stated that:

> "This experimentation is crucial for advancing sustainable pest management in the United States and globally. The release of sterile male insects, often referred to as the "sterile insect technique" is a proven integrated pest management strategy. It has no non-target effects, is directed at a very dangerous invasive species of medical and veterinary importance. It is a vector of pathogens that cause devastating diseases in humans and animals, e.g., Zika, dengue, chikungunya, yellow fever, and animal heartworm. Moreover, the Oxitec technology produces and releases sterile male Aedes aegypti mosquitoes that do not persist in the environment. I have conducted research on the production and use of sterile insects for about 50 years, including the screwworm, tropical fruit flies and mosquitoes. I know of no reason to not grant this EUP." (0375 p. 1)

Norman C. Leppla, University of Florida, added that:

> "Oxitec's technology has been thoroughly and independently studied for more than 20 years and has been the subject of more than 100 scientific articles 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 a decade of effort working through the U.S. regulatory system, which resulted in the U.S. FDA's Finding of No Significant Impact in 2016 and a 2020 EUP approval from your agency." (0375 p. 1)

Dan Killingsworth stated that:

> "The Oxitec sterile insect technique has proven itself effective in *Aedes aegypti* population reduction in previous trials and is highly suited for use in the fight to help protect public health within the United States." (0392 p. 1)

A coalition of organizations addressing mosquito control stated that:

> "Oxitec technology has already been tested in several countries and the first field deployment of OX5034 achieved up to 95% suppression of target disease-transmitting mosquito populations in dense urban settings in Brazil." (0393 p. 1)

Michael Weissmann stated:

> "Oxitec has developed a targeted, pesticide-free, safe biotechnology that has the potential to substantially reduce and, in some locations, likely eliminate populations of Aedes aegypti mosquitoes. Trials in South America and currently in the Florida Keys show great promise for the technique." (0459 p. 1)

EPA Responses to Comments in Unit VII.A. – Comments Questioning Whether OX5034 Would be Efficacious. With regard to comments equating efficacy to reduction in disease frequency, EPA's

authority to regulate the OX5034 mosquito under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) includes regulation of pesticidal claims regarding population control for the target pest. Claims that the OX5034 mosquito control or reduce "disease" would be regulated by the U.S. Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act (FFDCA). Oxitec, Ltd.'s product claims relate to population suppression, and not disease suppression. For more information, please see "EPA Response to Unit XII.F., Comments Challenging EPA's Interpretation That FIFRA Can Be Used to Regulate OX5034 Mosquito," and Unit XII.G., "EPA Response to Comments Arguing That EPA Needs to Develop New Regulations for GE Insects" of the Response to Comments document associated with the original EUP issued in April 2020 (EPA-HQ-OPP-2019-0274-0355).

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, that product (OX513A), Oxitec's first-generation product, is not part of the EUP testing at issue here. The application to amend and extend the EUP issued in 2020 is for OX5034, Oxitec's second-generation product. OX5034 differs from OX513A in that OX5034 has been engineered to only allow male offspring to survive into adulthood.

Further, in the present case, this extension and amendment of the 2020 EUP is issued under Section 5 of FIFRA to allow the generation of information necessary to ultimately support a registration application under Section 3 of FIFRA for OX5034, i.e., for the purpose of *determining* efficacy. Section 5(a) of FIFRA, 7 USC § 136c(a), states, in part, that EPA "may issue an [EUP] only if [EPA] determines that the applicant needs such permit in order to accumulate information necessary to register a pesticide under [FIFRA section 3]." While methods and results developed in countries other than the United States for determining efficacy may be helpful for developing testing protocols (and may be relevant to an ultimate registration decision under Section 3 of FIFRA), the results of those studies are not determinative to EPA's decision to issue an EUP under Section 5 of FIFRA for development of such data within the United States. EPA does not rely on opinions or reports in the press in forming its decision concerning the issuance of an EUP; rather the Agency relies on data and peer reviewed information in the literature.

EPA Responses to Comments in Unit VII.B -- **Comments Arguing for Alternative Approaches.** With regard to comment arguing that other control methods would be preferable, e.g., use of *Wolbachia*-infect mosquitoes, please see "EPA Responses to Comments in Unit IX.B. —- Potential Effect of Integrated Pest Management on OX5034 Efficacy" and "EPA Responses to Comments in Unit IX.C. - Comments Arguing for Alternative Approaches" of the Response to Comments document associated with the original EUP issued in 2020 (EPA-HQ-OPP-2019-0274-0355).

With regard to comments expressing concern that use of OX5034 would increase the number of matings occurring in *Ae. aegypti* populations and arguing that such increases would be novel for pesticidal programs, such increases would not be unique to use of OX5034 to control pest organisms. OX5034 can be considered a form of Sterile Insect Technique (SIT), a control method that has been used successfully

for decades for the control of various pests including mosquitoes. SIT depends on matings occurring between the sterile SIT males and wild females. SIT involves mass breeding of large quantities of target insects in a "factory," subsequent separation of the males from the females, followed by sterilization of the males by exposing them to low doses of radiation. These sterile males are then released to the wild where they mate with wild females. Control through use of SIT involving the release of irradiated male insects has been used for the control of a number of species, including several mosquito species. The purpose of the request to amend and extend the 2020 EUP is to continue to evaluate the next generation SIT approach developed by Oxitec, Ltd., i.e., OX5034, and to determine if it will be efficacious. With regard to the comment expressing concern about the possibility that the female lethal trait engineered into OX5034 might fail, Oxitec is required to monitor the test area and should failure be detected, Oxitec, Ltd., is required to terminate the experiment and initiate mitigation methods for the released OX5034 mosquitos.

EPA Responses to Comments in Unit VII.C -- Comments Arguing That OX5034 is Likely Efficacious. With regard to comments arguing that OX5034 will prove efficacious, please refer to the following section, "EPA Responses to Comments in Unit IX.D. - Comments Arguing that OX5034 is Efficacious" and "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" of the Response to Comment document associated with the original EUP (EPA-HQ-OPP-2019-0274-0355). In today's Response to Comment document, as described in Unit III., twenty-one comments supported issuance of the EUP, with these comments sharing the opinion that OX5034 would be efficacious in the field. Since Oxitec's EUP application has met FIFRA standards, the company is afforded the opportunity to determine whether OX5034 is efficacious in the field.

VIII. Comments on Trial Parameters

Some comments (0383, 0389, 0396, 0397, 0401,0413, 0426, 0428, 0429, 0461) offered recommendations on appropriate design for the testing protocol that would be implemented under the EUP. These comments revolved around: (1) concerns of the potential for OX5034 mosquitoes to move away from the test area; and (2) suggestions on specific aspects of trial parameters.

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

Comments in this category revolved around: (1) the relationship between *Ae. aegypti* dispersal distance and potential sources of tetracycline; and (2) potential consequences of imposing inadequate buffer zone requirements on the test areas.

1. Comments on potential for *Ae. aegypti* to move outside of the test area.

Some comments (0397) focused on the potential for *Ae. aegypti* to disperse from the test area.

GMO Free Florida contested EPA's conclusion 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* was inaccurate because it was based on an OECD document that did "not include the references provided . . . which show distances much greater than 200 meters. In fact, Oxitec's own study concluded that OX513A had a flight potential of 1490 meters." [Footnotes Omitted] [Emphasis in Original]

Further, GMO Free Florida voiced the opinion that:

> "Since Oxitec states, "*their dispersal by spontaneous flight is less than 200 m*" it would seem they are unaware of the observations that *Aedes aegypti* can travel more than 800 meters and therefore they must reassess based on this new information and increase the buffer zone and trap placement to at least greater than 2,500 meters." (0397 p. 16) [Footnotes Omitted] [Emphasis in Original]

GMO Free Florida contended that the conditions EPA placed on the EUP that *"Treated areas will be located greater than 400m from commercial citrus growing areas"* and *"BG Sentinel traps will be spread out to a maximum distance of 400m"* from the treatment area are inadequate because:

> ". . . some studies observed *Aedes aegypti* traveling 800 meters, as much as 1000 meters across water, up to 2,500 meters in some cases and when coupled with ocean winds *Aedes aegypti* could travel up to 3 1/2 miles to shore, this distance is not adequate to stop potential immigration of other *Aedes aegypti* into the treatment area or Oxitec's mosquitoes from immigrating into the untreated comparator area. This does not even include the fact that the Oxitec mosquitoes or other mosquitoes could still enter a vehicle and travel via vehicle from one area to another." (0397 p. 16) [Footnotes Omitted]

Challenging the Oxitec claim that " *the mosquito in any life stage cannot survive due to the high salinity of the waters*", GMO Free Florida pointed out that:

> "In the 1960's the U.S. military demonstrated that when coupled with ocean winds *Aedes aegypti* could travel up to 3 1/2 miles to shore." (0397 p. 15) [Footnotes Omitted]

And:

> " . . . despite Oxitec's misinformation, *Aedes aegypti* are fully capable of breeding in water with high levels of salinity. *Aedes aegypti* eggs may stay viable and resistant to desiccation for up to 450 days. If OX5034 mosquitoes, or the female they mate with, escape the test area there is a

> good likelihood they would successfully mate and/or deposit eggs in other areas." (0397 p. 15) [Footnotes Omitted]

GMO Free Florida, arguing that "[M]osquitoes are believed to frequently travel long distances via boat, automobile etc." stated that:

> "It is believed that the recent presence of *Aedes aegypti* in California was caused by commerce via air, railroad, or trucks traveling from the southern U.S. Even *Aedes aegypti* found as far away as the Netherlands are believed to have traveled there in airplane tires arriving from southern Florida. With over 3 million visitors to the Florida Keys a year, and numerous vehicles traveling in and out of the area, these genetically engineered mosquitoes escaping the test area is an extremely likely scenario." (0397 p. 15) [Footnotes Omitted]

Challenging the Oxitec claim that *"Geophysical containment is provided by the island location of the release site, where the site is predominantly surrounded by ocean,"* GMO Free Florida stated that:

> "Islands in the Florida Keys are not isolated island locations and California is not an island. The Keys are connected via roadway to the state of Florida, which is then connected via roadway to the rest of the continental U.S. So for the question of *"Will the genetically engineered(GE) mosquitoes travel outside of the Keys?"* The answer is an almost definite *"Yes"*. . . . This distance could easily place a genetically engineered mosquito in a vehicle intended for another state or another country." (0397 p.15) [Emphasis in Original]

Coupled with concerns that *"Aedes aegypti* can escape the trial area," GMO Free Florida reminded that *Aedes aegypti*:

> *". . . ,* are routinely found indoors and can survive cold weather, survive to adulthood in water with high salinity and have eggs that can resist desiccation. Oxitec's own study suggests genetically modified mosquitoes may travel as much as 1,490 meters." (0397 p. 15)

2. <u>Potential for *Ae. aegypti* to encounter tetracyclines</u>

Some comments (0383, 0396, 0397) in this category discussed potential environmental sources of tetracyclines.

Contesting EPA's conclusion that OX5034 mosquitoes are unlikely to survive even if dispersed beyond the test area, GMO Free Florida argued that:

> "This ignores that the OX5034 trait is self-limiting only in the absence of tetracyclines. If the OX5034 mosquitoes are dispersed to an area where tetracyclines are used these OX5034 will not only remain in the environment, but females will also be released." (0397 pp. 16-17)

GeneWatch UK and Center for Food Safety, pointing to EPA's finding that "'*most*' septic tanks in Florida are now gone (Response to Comments, p. 45) and other sources of tetracycline (e.g. cat feed, animal waste) not plausible (Response to Comments, p.46)" stated that:

> "However, concerns about potential exposure to tetracycline are sufficient for conditions to have been applied to the Florida trial in an attempt to limit such exposure – for example, Oxitec's Protocol (EPA-HQ-OPP-2019-0274-0358) states that, "*The outer boundary of the trial area (denoted by the traps furthest from the central release point) will be greater than 500 m from commercial citrus growing areas and from sewage treatment plants*". It is a matter of serious concern that no information has been supplied regarding potential tetracycline exposure at or near proposed release sites, particularly in the new proposed sites in California, including in commercial citrus growing areas or in septic tanks." (0383 p. 6; 0396 p. 5) [Emphasis in Original]

3. Comments suggesting potential consequences of *Ae. aegypti* dispersing from the test areas

Some comments (0396, 0397) speculated on potential consequences that could result from inadequate buffer zone requirements.

GMO Free Florida contested EPA's finding that OX5034 is not expected to establish should they disperse from the test area, stating that:

> "The EPA argues that, *"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 Ae. aegypti population regardless of whether that population is within or outside of the test area."* This ignores that the OX5034 trait is self-limiting only in the absence of tetracyclines. If the OX5034 mosquitoes are dispersed to an area where tetracyclines are used these OX5034 will not only remain in the environment, but females will also be released." (0397 pp. 16-17) [Emphasis in Original]

GMO Free Florida and Center for Food Safety contested Oxitec's claim that there would be no consequences should OX513A or OX5034 move out of the test area stating that:

> "Oxitec claims, "*Risk of establishment or spread has been determined to be negligible. The trial is short in duration and any unanticipated adverse effects are unlikely to be widespread or persistent in the environment. Most importantly, the status of the environment is restored when releases are stopped (i.e., the released mosquitoes all die, and the environment reverts to the pretrial status).*" While this claim was made about OX513A it is also applied to OX5034. There is

> no data provided to support this claim, hence it is an unsubstantiated claim at best and cannot be assumed to be true without data." (0396 p. 7; 0397 p. 24 & 26) [Footnotes Omitted]

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

Comments in this category revolve around: (1) buffer zone requirements, (2) monitoring, (3) eradication plans, and (4) the goal of the trial releases.

1. Comments on buffer zone requirements

Some comments (0396, 0397, 0461) expressed concern about the adequacy of EPA's buffer zone requirements.

Institute for Responsible Technology stated that:

> "Introducing GE mosquitoes should never be treated as a local, containable event. It's a global issue that can impact public health and the environment." (0461 pp. 1-2)

The Center for Food Safety stated that "500m is too small a distance" and suggested that:

> ". . . no mosquitoes should be released within a mile of known operations using tetracycline as a spray or feeding it to animals." (0396 p. 6)

GMO Free Florida expressed the opinion that a 500 meter buffer zone is inadequate:

> "It is possible for the females that mate with the OX5034 or the OX5034 males to leave the test area and travel to an area such as a citrus grove in Florida where oxytetracycline is sprayed on citrus crops. The current test area has a 500 meter buffer zone. Data from peer reviewed studies indicate that Aedes aegypti can fly significantly further than 500 meters, are routinely transported by vehicles and Aedes aegypti from South Florida have traveled as far away as the Netherlands." (0397 p. 19) [Footnotes Omitted]

2. Calls for public health surveillance

Three comments (0397, 0401, 0461) called for some type of public health surveillance being part of OX5034 testing.

The Institute for Responsible Technology and Californians for Pesticide Reform stated that:

> "No human clinical trials and no public health surveillance related to GE mosquito bites exist. The cause of any associated health problems could therefore go unnoticed. It would require

> large-scale outbreak of a serious reaction for health authorities to even mount an investigation, let alone consider the mosquito as a potential source." (0401 p. 2; 0461 pp. 4-5)

GMO Free Florida contended that:

> "Also, allergen databases are often incomplete and therefore the risk of an allergic response in residents exposed to the GE mosquitoes is a possibility and residents must be informed of and consent to such a risk. If all residents in the test area do consent Oxitec must provide a physician, as a part of the test, who will monitor the health of the residents that are exposed to Oxitec's mosquitoes." (0397 p.7)

3. Comments on eradication plans

Some comments (0396, 0397) argued that eradication plans should be in place.

GMO Free Florida and The Center for Food Safety stated that:

> "An immediate response plan to eradicate the mosquitoes must also be in place since the lethality trait cannot be fully relied on considering 50%, all male, offspring can survive and an even greater percent when they exposed to pet food, a likely scenario, or environmental tetracyclines." (0396 p. 9; 0397 p. 7) [Footnote Omitted]

GMO Free Florida contended that:

> "In the case of an adverse event being reported during this trial Oxitec must have a plan in place to recall the mosquitoes and/or evacuate the residents. This would involve erecting temporary structures outside of the test area, in case of an adverse event being reported, to evacuate residents to." (0397 p. 7)

4. Comments questioning the goal of the EUP testing

Several comments (0383, 0396, 0399, 0412, 0413, 0428) were received on the goals of the testing. Some comments (0383,0399, 0412, 0413, 0428) questioned the goals Oxitec has set for the trial. Some (0396, 0428) questioned why an expansion of testing was necessary. Another (0383) questioned whether Oxitec actually intends to measure population suppression.

GeneWatch UK contended the trials were not intended to show suppression of the *Ae. aegypti* population because:

> " . . . , it is not clear that the applicant has any intention of measuring population suppression of wild *Aedes aegypti* mosquitoes (let alone competitor species, see Section 4.3), nor that the EPA

> intends to evaluate this. "*Mortality rates will be evaluated by comparing rates of survival to adulthood between treated female larval progeny (those fathered by OX5034 males) and untreated female larval progeny (those fathered by wild males)… Mating fraction data will also be collected under the proposed field trial protocol, as requested by EPA, but as described here and elsewhere, efficacy is most appropriately defined by the effect (percentage mortality) of the pesticide on treated individuals, compared to untreated individuals*" (Oxitec's trial protocol, p. 4, EPA-HQ-OPP-2019-0274-0358). And further, "*Efficacy data analyses are subject to ongoing discussions with EPA*" (Oxitec's trial protocol, p. 21, EPA-HQ-OPP-2019-0274-0358). This means that it is Oxitec's intention merely to show that the progeny of its released GE mosquitoes have lower survival rates than non-GE mosquitoes. This is not sufficient to show any suppression effect of the wild adult female *Aedes aegypti* population (which bite and spread disease). Further, even if mating fraction is calculated, this also does not demonstrate that the population density of biting adult female mosquitoes has been reduced." (0383 p. 2) [Emphasis in Original]

Casey Schlinker stated that:

> "The testing sites increased acreage seems unnecessary. . . . If testing has already taken place what more needs to be done to get the results necessary by extending an additional 24 months." (0428 p. 1)

The Center for Food Safety stated that:

> "Under a 2-year Experimental Use Permit, Oxitec was granted in the spring of 2020, permission to release over 1 billion genetically modified mosquitoes across 6,600 acres in Florida and Texas. . . . Without releasing any data from the Florida trial, Oxitec wants to expand to California. It proposes to release 30,000 males per acre per week over this large scale (with no explanation as to why this is higher than used in Florida), which equals the potential release of more then [sic] 2.5 billion mosquitos per week in the state. This hardly seems like a small experimental trial. Although no information is provided about the specific counties where these releases would occur, Oxitec's application indicates it could be as many as 20 counties, covering a significant portion of the state." (0396 p. 2)

5. <u>Comments on environmental monitoring</u>

Several comments (0389, 0397, 0413, 0426, 0429, 0461) offered opinions on monitoring. Some of the comments expressed concern that monitoring by EPA was non-existent or inadequate. Other comment (0389), however, supported the provisions EPA imposed on the testing.

Anonymous (0429) stated that:

> "I would consider performing the experiment where it is most relevant because the testing will be more accurate and if the tests prove to be accurate, they are already in the correct area. . . . If the U.S. does not need the results as much as another area of the world, why would we perform the experiment here rather than there? I do believe that this should be licensed and regulated by necessary means in order to perform the experiment and further actions properly." (0429 p. 1)

GMO Free Florida stated that:

> *"Aedes aegypti* eggs may stay viable and resistant to desiccation for up to 450 days. There is no mention of post-trial monitoring of potentially viable eggs for this duration after the trial." (0397 p. 37) [Footnotes Omitted]

The Institute for Responsible Technology contended that:

> "EPA has no plans for long term monitoring, no assignment of liability in case things go wrong, no way to confirm that the data from Oxitec submitted correct data before the trial, and no way to confirm its claims after." (0461 p. 8)

GMO Free Florida stated that:

> "No monitoring of animal health during the releases is being done and therefore adverse effects to animals as a result of this experiment would go unnoticed." (0397 pp. 21 & 25)

Kimberly Sikora expressed concern that "[N]o one is monitoring Oxitec in the Florida Keys but Oxitec" and "[T]here is no accountability, no entity overseeing their experiment in our fragile Florida Keys Environment" in light of information shared by Oxitec:

> "The Materials made available by Oxitec include BIOLOGICAL Materials that are EXPERIMENTAL in nature, and Oxitec makes NO REPRESENTATION and gives NO WARRANTY as to the PERFORMANCE of the Materials in any particular manner, that they are FIT FOR ANY PARTICULAR PURPOSE or that the materials will NOT HAVE ANY LATENT or OTHER DEFECTS." (0426 p. 1) [Emphasis in Original]

John Ulloth stated that OX5034 is:

> " . . . impossible to track, or recall specimens! Impossible to stop." (0413 p. 1)

GMO Free Florida stated that EPA should consider the effect of mating competition:

> "This is especially important since the increase in males will increase mating competition and potentially drive already existing *Aedes aegypti* from the treatment area into the untreated comparator area which would skew the results in favor of reduction in the treatment area." (0397 p. 16)

On the other hand, AMCA (0389) stated that:

> "AMCA also understands the proposed amendment to the EUP will continue significant protections, including weekly monitoring and sampling of the mosquito population in the treatment areas, enabling the Agency to determine the product meets the FIFRA safety standard. Additionally, it's important to note that only the adult male self-limiting mosquitoes will be present in the environment and they do not bite people, further supporting EPA's finding that the mosquitoes are not expected to present risks to human health." (0389 p. 1)

EPA Response to Comments in Unit VIII.A - 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, potential for dispersal of OX5034 was assessed in the following section "EPA Response to Unit X.D. - Comments on the Potential for Mosquitoes to Move Away from the Test Area" of the Response to Comments document associated with the 2020 EUP (EPA-HQ-OPP-2019-0274-0355), as well as in the 2020 (EPA-HQ-OPP-2019-0274-0356) and current Section G[24] analyses which include containment measures that is also posted in docket EPA-HQ-OPP-2019-0274.

Regarding the comments requesting larger buffer zones between the OX5034 release points and potential environmental tetracycline sources, it is relevant to emphasize that EPA in its original assessment of the EUP concluded that even without any restrictions on the local trial area boundaries, the likelihood that OX5034 mosquitoes would encounter tetracycline levels high enough to result in OX5034 females is low. This assessment was based on known egg-laying preference of *Ae. aegypti* (e.g., tires, gutters) coupled with literature surveys of environmental concentrations of tetracycline analogues, which together indicated that levels in the environment are lower than those shown necessary through dose response testing of mosquitoes to rescue OX5034 females. Nevertheless, EPA decided to impose a conservative 500 m buffer zone to further increase confidence that there will be no OX5034 females in the trial areas and to reduce uncertainty surrounding potential degradation products. That buffer distance included 200 m for released OX5034 males + 200 m for mated *Ae. aegypti* females + 100 m of additional buffer. An updated literature survey of environmental tetracycline sources conducted as part of this EUP amendment and extension confirmed the results of the original assessment, showing that environmental tetracycline levels are not expected to be high enough to rescue OX5034 females (Unit III.B.2 of the Human Health and Environmental Risk Assessment for the EUP amendment/extension request). However, as before, EPA will be imposing a conservative buffer zone of 500 m between the release sites and any potential tetracycline sources determined to be

[24] EPA's review of the details of the proposed experimental program. Hereinafter referred to as the "Section G." Section G analyses can be found in the docket established for this action.

relevant to Florida and California. The Agency finds that this buffer distance remains protective, given the low likelihood for mosquitoes to encounter environmental tetracycline levels high enough to rescue female OX5034. In further response to the comments, it is also relevant to note that the Agency acknowledged in its original assessment that "*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 (OECD 2018[25]).*" (emphasis added). The articles cited by the commenters do not negate that assessment and thus, the Agency reaffirms its conclusion regarding the *Ae. aegypti* dispersal range.

As an additional conservative measure, the extension and amendment of the EUP will include additional adult mosquito trapping near tetracycline sources within 1 kilometer of any release site for the duration of the releases and the post-monitoring period. Individuals captured will undergo a PCR analysis for determination of the presence of the OX5034 cassette. If a female OX5034 mosquito is found, Oxitec, Ltd., will immediately cease releases of all OX5034 mosquitoes, as soon as practical apply adulticide and larvicide pesticides to the treated area where the surviving females were detected, and continue to monitor for the presence of the OX5034 genetic construct in female *Ae. aegypti* until OX5034 mosquitoes are no longer found for at least two successive mosquito generations, a minimum of 10 weeks.

With regard to the risk of allergenicity or toxicity from the saliva of female mosquitos, please see the following section "EPA Response to Comments in Unit VII.B. - Comments on Toxicity/Allergenicity of Proteins Engineered into OX5034" of the Response to Comments document associated with the original EUP (EPA-HQ-OPP-2019-0274-0355). Additionally see, "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" of the same document, which demonstrates that the risk of tetracycline rescue, and thus the presence of biting female OX5034 mosquitos, is negligible.

EPA Response to Comments in Unit VIII.B. - Comments Offering Suggestions on Specific Aspects of the Trial Parameters. With regard to comments that physicians should monitor the health of human residents within the trial sites, please see "EPA Response to Unit XII.I. - Comments that EPA Must Address Ethical Concerns: Human Subjects and Informed Consent" of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274).

With regard to comments requesting that an eradication strategy be in place prior to testing, please see "EPA Response to Unit XII.H. - Comment that EPA Should Ensure Post-Release Control Measures" of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274), as well as the Section G analysis and issuance letter for the current EUP in the same docket. If at any time during the course of the EUP, Oxitec, Ltd., finds female individuals containing the OX5034 genetic

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

construct surviving to adulthood, Oxitec, Ltd., must immediately cease releases of all OX5034 mosquitoes, as soon as practicable apply adulticide and larvicide pesticides to the treated area where the surviving females were detected and continue to monitor for the presence of the OX5034 genetic construct in female *Ae. aegypti* until OX5034 mosquitoes are no longer found for at least two successive mosquito generations, a minimum of 10 weeks. EPA may require additional applications of adulticides and larvicides if fluorescent mosquitoes continue to be found in the treated area after the initial detection.

With regard to comment stating that the incorrect parameters are being assessed to measure efficacy, as stated by the commenter, EPA has reviewed the experimental protocols and Oxitec, Ltd., is expected to provide data that may be supportive of a Section 3 registration. With regard to the comment questioning whether the amount of acreage requested is needed, EPA assessed the justification for the acreage amount and locations requested in the Section G analysis, titled "Review of the Section G (dated February 18 2022) for an Experimental Use Permit (EUP) 93167-EUP-2 to Test OX5034 *Aedes aegypti* Mosquitoes in California and Extend the Existing EUP in Florida" that is included in the docket (EPA-HQ-OPP-2019-0274). Further, regarding the comment questioning why additional testing is necessary if testing was already conducted under the previous EUP approval, EPA notes that Oxitec, Ltd., may apply for amendments and extensions of their EUP at any time. Such amendments or extensions may be approved by EPA if the request is justified and the risk assessment meets the standards of FIFRA. See also Unit VII.C - Comments Arguing That OX5034 is Likely Efficacious above for additional details. EPA has evaluated the amendment and extension of this EUP in the context of the capacity of the data to support Section 3 registration.

With regards to comments suggesting other areas for OX5034 test locations or refuting the necessity of the current test locations, please see "EPA Response to Unit X.C. - Comments Suggesting That Testing Be Performed in Areas Others Than Those Proposed in the EUP Application" of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274).

Regarding comments suggesting possible tetracycline rescue in other locations, please see "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" of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274).

With regard to comments that there is no post release monitoring, please see "EPA Response to Unit XII.H. – Comment that EPA Should Ensure Post-Release Control Measures" of the Response to Comments document associated with the original EUP (EPA-HQ-OPP-2019-0274). Note, there is post-release monitoring for OX5034 mosquitoes, such that following the last observed fluorescent larvae, Oxitec, Ltd., will continue trapping and searching for fluorescent individuals for a minimum for 10 consecutive weeks (see, Section G analysis, titled, "Review of the Section G (dated February 18, 2022)

for an Experimental Use Permit (EUP) 93167-EUP-2 to Test OX5034 *Aedes aegypti* Mosquitoes in California and Extend the Existing EUP in Florida.").

With regard to the possibility that the releases would drive wild *Ae. aegypti* males from the treatment area into the untreated area and skew the results in favor of reduction in the treatment area, please see, Section G analysis, titled, "Review of the Section G (dated February 18, 2022) for an Experimental Use Permit (EUP) 93167-EUP-2 to Test OX5034 *Aedes aegypti* Mosquitoes in California and Extend the Existing EUP in Florida," which describes the measurements collected as part of the EUP. Because measurements include the adult over-flooding ratio or sex ratio achieved (i.e., Oxitec males:wild male ratio and/or male:female ratio), EPA would be able to detect whether the situation described by the comment occurred (i.e., the male:female ratio in the untreated area would be higher than expected). However, it is also important to note that EPA does not expect the scenario as described by the commenter to occur.

IX. Comments Calling for Additional Testing and Information

A number of comments (0372, 0380, 0383, 0396, 0397, 0400, 0401, 0410, 0412, 0413, 0429, 0432, 0439, 0444, 0451, 0452, 0456, 0461, 0394) were received calling for additional testing. Some of these comments offered specific observations on what the commenter believed was needed. Other comments (0399, 0410) simply expressed the opinion that not enough was known about OX5034 or that EPA did not require or review any data before approving the EUP.

A. Comments on Reproducibility of Studies by Independent Experts

Six commenters (0396, 0397, 0401, 0408, 0450, 0461) argued the importance of, and need for, independent experts to reproduce and verify and data developed by Oxitec.

GMO Free Florida stated that:

> "Since reproducibility is one of the main principles of the scientific method, these studies must be reproduced by independent experts. This can be done through programs such as PLOS ONE's Reproducibility Initiative. Once Oxitec has conducted these necessary studies and they are replicated by independent experts, then Oxitec must reassess and include this new information. Until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe." (0396 pp. 7 & 8; 0397 p.8, 19, 20, 24, 26, 27, 28, 31 & 32) [Footnote omitted]

Maggie Mistal requested that:

> "I urge the EPA to look at independent data from past testing locations to ensure this technology is safe and effective in the short and long term. To introduce a GMO mosquito into

> the environment is not something to be taken lightly especially when other options exist." (0408 p. 1)

Ariane Glazer stated that:

> " . . . the people of this country need sound proven 3rd party long term safety studies by independent scientists before we release an unknown entity like a GM mosquito into nature!!" (0450 p.1)

Contending that the "U.S. EPA appears to be operating under this myth of predictability and safety," Californians for Pesticide Reform and the Institute for Responsible Technology argued that:

> "Thus, they don't require GMO companies to conduct a thorough evaluation, even of changes in the genome, let alone those in the RNA, proteins, and metabolites." (0401 p. 3; 0461 p.7)

The institute for Responsible Technology further argued that:

> "With long-term and potentially irreversible consequences, the decision to release engineered insects into the wild should be preceded by the most exhaustive independent scrutiny, using state-of-the-art scientific tools and inputs from a host of experts." (0461 pp. 1-2)

Florida Keys Environmental Coalition stated that:

> "When Oxitec claims all females die and it is flawless, how far has that been tested, through how many generations and are these Oxitec claims, or have they been double blind replicated as is more of a standard practice that just accepting the vendors word for it?" (0400 p. 8)

GMO Free Florida stated that Oxitec:

> ". . . must also provide the lowest dosage recorded to allow a female to survive to adulthood so that independent scientists can attempt to replicate their results." (0397 p. 19)

B. Calls for Additional Information or Testing that are Specific to the Experimental Area

1. <u>Calls for additional information or testing on offspring of OX5034 males and local wild *Ae. aegypti* females</u>

Comments received on this category (0397) argued that additional testing should occur using offspring resulting from OX5034 male mosquitoes and female mosquitoes from the experimental areas. Arguments revolved around claims that vectorial competence can vary between *Ae. aegypti* populations.

GeneWatch UK, noting that the "*background of the LWT strain is comprised of genetics from ten separate Ae. aegypti colonies . . . established from mosquitoes that were collected in the Mexican State of Chiapas in 2006 (Wise de Valdez et al. 2011,)*" stated that:

> "It remains unclear why the EPA considers it lawful or safe to release a Mexican strain of *Aedes aegypti* mosquito in the USA without even testing its vectorial capacity. Introducing an Aedes aegypti strain with increased vectorial capacity for any disease (dengue, zika, chikungunya) could have serious and irreversible repercussions for public health. . . . It is hard to see how this risk can have been properly assessed without testing the vectorial capacity of the introduced strain and any potential admixed strains." (0383 pp. 4-5)

Friends of the Earth stated that:

> "The vectoral capacity of Oxitec's GE mosquitoes should be fully assessed, as well as the potential vectoral capacity of hybrid mosquitoes that could carry the genetic material from Oxitec's GE mosquito. This information should be made publicly available ahead of a public comment period." (0394 p. 3)

GMO Free Florida stated that:

> "A recent study indicates that a different genetically modified mosquito was better able to transmit malaria. Which means that some genetic modifications to mosquitoes could cause greater transmission of mosquito-borne diseases, thus increasing the number of cases of human diseases such as dengue fever, Zika virus or chikungunya or even animal diseases such as canine and feline heartworm or avian malaria that could impact the endangered or threatened Southern bald eagle, Roseate tern, Everglade snail kite, Cape Sable seaside sparrow, Bachman's warbler, Wood stork, Piping Plover and Red knot in Monroe county, Florida. Therefore, Oxitec cannot rely on vectorial capacity for only the host species OX5034 before transformation, and. . . disease transmission testing, including transovarial transmission and venereal transmission testing, must be done on the transformed OX5034 and the offspring of transformed OX5034 x wild *Aedes aegypti* present in the Florida Keys and California to determine the vector competence." (0397 p. 9) [Footnote omitted]

And:

> "Oxitec must also conduct studies for all mosquito-borne diseases *Aedes aegypti* is a vector for with regards to transovarial transmission of OX5034 x wild *Aedes aegypti* in the Florida Keys and in California compared to wild male x wild female *Aedes aegypti* in these locations." (0397 p. 31)

2. Potential for resistance in different genetic backgrounds

Three commenters (0383, 0397, 0400) pointed to the potential effects of the genetic background as a possible mechanism for resistance development to the female-lethal trait.

GMO Free Florida argued that "male OX5034 x wild Florida Keys and California *Aedes aegypti* offspring must be tested for female offspring that may survive to adulthood" and not just crosses between laboratory strains because:

> "Another possibility is that target pest populations may have genetic background components which provide resistance to lethal systems." (0397 p. 32) [Footnote omitted]

Arguing that a "recent study indicates that a different genetically modified mosquito was better able to transmit malaria," GMO Free Florida, stated that:

> " . . . male OX5034 x wild Florida Keys and California *Aedes aegypti* offspring must be tested for female offspring that may survive to adulthood, and not just a reliance on male OX5034 x laboratory strain *Aedes aegypti*, or wild *Aedes aegypti* from areas outside of the Florida Keys and California. An increase in mosquitoes and mating would likely lead to an increased risk of mosquito-borne diseases." (0397 p. 32). [Footnote omitted]

GMO Free Florida stated that:

> "The claim of no female offspring surviving to adulthood is based on past breeding experiments and not experiments using the wild *Aedes aegypti* found in the Keys or California. It is possible that when Oxitec males breed with wild *Aedes aegypti* females found in the Keys or California, it can alter the amount of female offspring that survive as target pest populations may have genetic background components which provide resistance to lethal systems. Therefore, Oxitec must conduct studies to determine the amount of female offspring that survive to adulthood when Oxitec males breed wild *Aedes aegypti* females found in the Keys and California. […] Oxitec has failed to realize that if the lethality trait fails that would mean a greater potential number of *Aedes aegypti*, both male and female, since Oxitec would have released millions of *Aedes aegypti a*nd these mosquitoes will successfully mate and have offspring that survive to adulthood." (0397 pp. 20 & 32) [Footnote omitted]

The same commenter further argued that "[T]etracycline levels required for female offspring of OX5034 males mated with wild females, in the Keys or California, to survive to adulthood may be lower than OX5034 bred with lab strains," further stating:

> "Oxitec appears to be speculating as to the amount of tetracycline required based on past breeding experiments and not experiments using the wild *Aedes aegypti* found in the Keys or

> California. It is possible that when Oxitec males breed wild *Aedes aegypti* females found in the Keys and/or California it can alter the amount of tetracycline required for the female offspring to survive to adulthood. Therefore, Oxitec must conduct studies to determine the amount of tetracycline that would be required for the offspring to survive to adulthood when Oxitec males breed wild *Aedes aegypti* females found in the Keys and California." (0397 p. 19)

Florida Keys Environmental Coalition, quoting the paper by Knudsen et al [26] that stated that "[O]ur data suggest that standing genetic variation in an insect population could provide multiple mechanisms for resistance to the tTA overexpression system," stated:

> "Why is there no resiliency testing of the fluorescent marker? […] The importance of this can be seen in a study published by Knudsen, et. al. Genetic Variation and Potential for Resistance Development to the tTAV Overexpresion Lethal System in Insects, that shows variation occur in seeming homogeneous subspecies, such as FL Keys Aedes Aegypti, or Almeda County CA Aedes Aegypti, where the lethality ranged from 11% to 97% and is attributed to the broad spectrum of underlying differences, . . . In no way is this tTAV feedback loop system as efficient and absolute as Oxitec claims their [sic] is, but the importance is that yet again another research paper other and Zhao, suggest genetic variation in the wild population has an effect on the reliability, or resiliency of the lethality." (0400 p. 8)

GeneWatch UK stated that:

> "In addition to the information in our previous submission, a new reference in relation to the development of resistance should be taken into consideration. This paper adds important evidence to support the view that evaluation of resistance should be done (on a large scale) in contained use, before any release." (0383 p. 6) [Footnote Omitted]

3. <u>Arguments for additional testing based on the possibility that there may be sufficient tetracycline sources to allow female OX5034 mosquitoes to mature to adults</u>

One commenter (0397) argued that additional testing should occur specific to the experimental areas to examine whether there may be sufficient tetracycline sources in or near the test areas to allow female OX5034 females to mature to biting adults.

GMO Free Florida argued that current levels of tetracyclines in the experimental areas should be tested:

[26] Knudsen, K.E., Reid, W.R., Barbour, T.M., Bowes, L.M., Duncan, J., Philpott, E., Potter, S. and Scott, M.J., 2020. Genetic variation and potential for resistance development to the tTA overexpression lethal system in insects. *G3: Genes, Genomes, Genetics*, *10*(4), pp.1271-1281.

> ". . . , preferably from peer reviewed studies, that recent samples of soil, pet food, septic tanks, cesspits, manure, etc. in the experimental areas have been tested for tetracycline, chlortetracycline, doxycycline, oxytetracycline, etc". (0397 p.19)

C. Calls for Additional Information or Testing for Toxicity or Allergenicity of OX5034 Transgenic Proteins

Three commenters (0396, 0397, 0400) called for additional testing of the OX5034 tTAV and DsRed2 proteins for toxicity or allergenicity. Two commenters (0396, 0397) called for testing to determine if there are differences in the allergen levels of the OX5034 GE *Ae. aegypti* compared to wild *Ae. aegypti* currently found in the Keys and California.

1. <u>Calls for additional information or Testing of the OX5034 tTAV and DsRed2 Proteins for Toxicity or Allergenicity</u>

Three commenters (0396, 0397, 0400) called for additional testing of the OX5034 tTAV and DsRed2 proteins for toxicity or allergenicity.

GMO Free Florida recommends testing of the OX5034-specific proteins for allergenicity, referring to the FAO/WHO guidelines for the assessment of foods derived from biotechnology, stating that:

> "According to A Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, *"Since identity of 6 contiguous amino acids has an appreciable risk of occurring by chance, verification of potential crossreactivity is warranted when criterion (1) is negative, but criterion (2) is positive. In this situation suitable antibodies (from human or animal source) have to be tested to substantiate the potential for crossreactivity"* Therefore, Oxitec must perform such tests to assess allergenicity." (0397 p. 8) [Footnote omitted]

And:

> "Sequences for tTAV and DsRed2 specifically from OX5034 must be made available so they can be put into allergen databases." (0397 p. 3)

With regard to the tTAV-OX5034 and DsRed2-OX5034 proteins, the Center for Food Safety stated:

> ". . . , no data is presented for OX5034. Even if Oxitec provided data for a few hundred or thousand OX5034, it is still possible that some unknown percent of OX5034 mosquitoes do have these proteins in their saliva. Therefore, toxicity and allergenicity studies must be conducted to determine what happens if people are bitten by OX5034 with the transgenic proteins expressed in their saliva." (0396 p. 8)

GMO Free Florida also argued that "toxicity and allergenicity studies must be conducted to determine what happens if people are bitten by OX5034," stating that:

> "Even if Oxitec provided data for a few hundred or thousand OX5034, it is still possible that some percent of OX5034 mosquitoes do have these proteins in their saliva." (0397 p. 8)

And:

> "Oxitec must conduct allergen tests of every person in the trial area to ensure they [sic] are not allergic reactions before releasing the millions of genetically engineered mosquitoes they plan to release. If someone in the test area is allergic this could cause a severe allergic reaction and a medical emergency. Oxitec cannot take risks with the health of the people in the trial area and all potential medical risks must be considered, including but not limited to testing every individual in the trial area for allergenicity potential." (0397 pp. 36-37)

Florida Keys Environmental Coalition stated that:

> "Oxitec has never clinically tested the bite of their GMMs on humans and the allergenicity analysis of the Oxitec protein sequences continues to be a diffused and incoherent discussion." (0400 p. 8)

GMO Free Florida stated that:

> "Oxitec was asked whether health studies were conducted on humans who were bitten by GE mosquitoes, they replied that many of the scientists working with the GE mosquitoes had been bitten and no adverse health effects were reported. So, all they have is anecdotal evidence from their own staff. This is insufficient when human health is potentially at risk." (0397 p. 7) [Footnote omitted]

Contesting EPA's conclusion that OX5034 mosquitoes are unlikely to survive even if dispersed beyond the test area, GMO Free Florida argued that:

> "This ignores that the OX5034 trait is self-limiting only in the absence of tetracyclines. If the OX5034 mosquitoes are dispersed to an area where tetracyclines are used these OX5034 will not only remain in the environment, but females will also be released. As the EPA has not evaluated the allergenicity or toxicity of saliva from OX5034 females this is a risk that has not, and must be evaluated by the EPA." (0397 pp. 16-17)

GMO Free Florida and the Center for Food Safety suggested that additional rodent and non-rodent studies should be conducted to determine the potential from accidental swallowing of OX5034, stating that:

> "Oxitec should also conduct feeding trials using rodents and non-rodents to assess toxicity as it may relate to humans, since humans may also accidentally swallow Oxitec's mosquitoes. The studies should also be for the life of the rodents and 48 months in duration for non-rodents and should not be limited to mortality, appearance, size, and behavior, as their previous studies are limited to, but should include examination of all major organ systems, include histological examination of organs as well as all other health parameters typical of chronic toxicity/carcinogenicity studies." (0396 p. 8; 0397 p. 26)

2. Mosquito Allergen Levels in OX5034 *Ae. aegypti* Compared to Wild *Ae. aegypti*

Two commenters (0396, 0397) called for testing to determine if there are differences in the allergen levels of the OX5034 GE *Ae. aegypti* compared to wild *Ae. aegypti* currently found in the Keys and California.

GMO Free Florida and the Center for Food Safety stated that:

> "According to the mutation rates some OX5034 females may be released and some people will be bitten. Since there are at least 8 allergens that have been found in *Aedes aegypti* saliva, an initial increase in the number of *Aedes aegypti* females could increase the number of allergic reactions in the Keys and California. . . . Oxitec also does not seem to indicate if there are differences in the levels of proteins in the saliva of the GE mosquitoes compared to wild mosquitoes in the Florida Keys or California. Since there are at least 8 allergens that have been found in *Aedes aegypti* saliva, an increase in these levels of allergens in GE mosquitoes may increase allergic responses or increase severity of allergic responses in people in the test area bitten by these GE female *Aedes aegypti*. Oxitec must therefore also conduct studies to determine if there are differences in the allergen levels of their GE *Aedes aegypti* compared to wild *Aedes aegypti* currently found in the Keys and California." (0396 p. 8; 0397 pp. 6-7) [Footnotes omitted]

3. Calls for testing on the tTAV-OX5034 and DsRed2-OX5034 proteins for toxicity

Two comments (0396, 0397) requested that the tTAV-OX5034 protein be tested for toxicity.

The Center for Food Safety argued that:

> "The proteins expressed by the transgenes may be toxic. Although Oxitec claims, "tTA and its variants, such as tTAV, have been used in fungi, rodents, plants, and mammalian cultures with no known non-target adverse effects on the environment or human health", signs of toxicity and neurotoxicity have been reported in mice expressing the tTA protein. Oxitec should therefore attempt to replicate these studies, finding toxicity and until Oxitec has conducted such studies

> and they are replicated by independent experts their GE mosquitoes cannot be considered safe." (0396 p. 9) [Footnotes omitted]

GMO Free Florida contested Oxitec's conclusion that the proteins are not toxic because although *"some potential symptoms of toxicity have been reported in transgenic mice expressing high levels of tTA or its variants (Whitsett and Perl, 2006) other papers have observed no apparent toxicity,"* arguing that the papers that do not observe toxicity are:

> ". . . not evidence to counter the potential symptoms of toxicity that have been observed. Oxitec must therefore reassess and include this new information as valid and not simply dismiss it. Oxitec should therefore attempt to replicate these studies finding toxicity and until Oxitec has conducted such studies and they are replicated by independent experts their GE mosquitoes cannot be considered safe." (0397 p. 27)

D. Calls for Additional Testing or Information on Environmental Considerations

Comments received (0372, 0383, 0396, 0397, 0383, 0401, 0432, 0452) revolved around requests for additional testing or information on the effects OX5034 mosquitoes might have on other organisms. Some of the comments (0396, 0397) stated the specific type of additional testing that commenters' argued should be done to examine potential effects on organisms that might consume mosquitoes and threatened or endangered species. Some (0396, 0397) comments called for long-term studies.

1. <u>Requests for Additional Testing or Information on the Effect of OX5034 on Other Organisms</u>

Some comments (0383, 0396, 0397, 0452) argued that additional testing on non-target organisms should be performed.

GeneWatch UK stated that:

> "No feeding trials have been done for mammals or birds (risk assessment, p.45-46), although Oxitec has undertaken some feeding trials for an 'aquatic invertebrate' (p.46, risk assessment) and with crayfish and guppies (p.48, risk assessment)." (0383 p. 7)

With regard to the 14 day acute toxicity study using *Poecilia reticulata* and a 96 hour study using *Pacifastacus leniusculus*, GMO Free Florida and the Center for Food Safety stated that:

> "These studies have little to no relevance for mammals, including humans that may consume or otherwise be exposed to OX5034." (0396 p. 8; 0397 p. 26)

Contesting EPA's conclusion that *"it does not appear that the salamanders . . . feed significantly on Ae. aegypti larvae,"* GMO Free Florida stated that:

> ". . . , larval feeding studies should be conducted before any further experimentation especially considering many of these larvae that will consume *Aedes aegypti* are endangered species. For example tiger salamander tadpoles are known for consuming large amounts of mosquito larvae and the California tiger salamander is an endangered species." (0397 p. 21) [Footnotes omitted]

GMO Free Florida argued that:

> "Toxicity testing with amphibians consuming OX5034 is absolutely necessary considering the risk to endangered species." (0397 p. 23)

GeneWatch UK stated that:

> "In addition, there is no information regarding local mosquito predators or prey at any of the sites. This is a particularly striking omission in the light of the proposal to undertake open releases of GE mosquitoes in a new state (California)." (0383 p. 7)

Discussing international regulatory bodies, GMO Free Florida and the Center for Food Safety stated that:

> "Oxitec told Olive Oil Times that Spain's National Biosafety Commission requested that predator studies be held. Oxitec stated they would conduct the studies requested. If Oxitec is willing to conduct these studies for Spain they must be willing to conduct them for the U.S. as well." (0396 p. 7; 0397 p. 24) [Footnote omitted]

Craig Zabransky questioned whether it is possible to determine whether the "science benefits out weight the risks" when:

> "NO studies were conducted to understand the effect on the local environment and its unique flora and fauna or species (some endangered)." (0452 p. 1) [Emphasis in Original]

Meagan Morrison Hill stated that:

> "The EPA required NO safety studies in its approval process of a wild release . . . The EPA requires NO reporting of data and set no standards for success or failure of this experiment." (0399 p. 1) [Emphasis in Original]

The Center for Food Safety, with regard to species in the testing areas, asked:

> " . . . , what happens if the transgene is consumed by any or all of these species? Nobody knows, because there have been no studies published on the subject. Without testing of actual species

> in the Keys and California counties where mosquitoes might be released, Oxitec is basing their assessment on speculation, not science." (0396 p. 7)

2. Calls for Specific Type of Additional Testing Including Long-Term Studies

Some commenters (0396, 0397) offered opinions on specific testing parameters including calls for long-term studies.

GMO Free Florida and the Center for Food Safety, noting that the Section G indicates that the "Season of Application" would be *"May-December (but could be deployed any time of year)*" stated that:

> "Oxitec has not provided adequate evidence to conclude there is no toxicity for insectivores in the Keys or California, especially for a duration of potentially all year for at least 24 months, and now even longer in Florida for potentially another 2 years. The only studies Oxitec has provided are a 14 day acute toxicity study using *Poecilia reticulata* and a 96 hour study using *Pacifastacus leniusculus*. These studies are of insufficient duration and include insufficient parameters to assess subchronic and chronic toxicity or carcinogenicity." (0396 p. 7-8; 0397 p. 25-26)

GMO Free Florida and the Center for Food Safety went on to argue that, given the potential duration of the EUP testing:

> "Oxitec must therefore perform toxicity studies using insectivores present in the Keys and California for a duration of at least 48 months, or the life of the subject if the subject does not live for 48 months. These toxicity studies should not be limited to mortality, appearance, size, and behavior, but should include examination of all major organ systems, including histological examination of organs as well as all other health parameters typical of toxicity studies. Multigenerational exposure, as well as transgenerational effects must also be considered since a large number of environmental factors have been shown to promote the epigenetic transgenerational inheritance of disease or phenotypic variation in a variety of different species, including humans. Until Oxitec has conducted such studies their GE mosquitoes cannot be considered safe for any insectivores in the Keys." (0396 pp. 7-8; 0397 p. 26) [Footnotes omitted]

Center for Food Safety expanded this statement to include California:

> "Multigenerational exposure, as well as transgenerational effects must also be considered since a large number of environmental factors have been shown to promote the epigenetic transgenerational inheritance of disease or phenotypic variation in a variety of different species, including humans. Until Oxitec has conducted such studies their GE mosquitoes cannot be considered safe for any mammals in the Keys or California." (0396 pp. 7-8) [Footnote omitted]

E. Testing and Antibiotic Resistance

Some comments (0397, 0400) called for additional testing to address the potential for OX5034 mosquito releases to increase antibiotic resistance in microbial populations.

GMO Free Florida stated that:

> "Tetracycline resistant bacteria could also be found on GE *Aedes aegypti* due to the potential for tetracycline resistant bacteria in the laboratory where tetracycline is used and GE *Aedes aegypti* are potentially exposed. Therefore, Oxitec must conduct studies to determine if tetracycline resistant bacteria are found on GE *Aedes aegypti* as well." (0397 p. 14)

Florida Keys Environmental Coalition asked:

> " . . . why the simple requests by near 3 dozen FL Keys doctors, to analyze this product for antibiotic resistant bacteria has not been done? This technology is going everywhere and in many forms, not just Aedes Aegypti mosquitoes, but moths and fruit flies and a myriad of other 'pests' that Oxitec will vanquish," (0400 p. 7)

F. Calls for Caged Trials Prior to Open Field Testing

Several comments (0383, 0394, 0396, 0397, 0400, 0406, 0452) called for Oxitec to perform caged trials prior to being permitted open field testing.

The Center for Food Safety stated:

> "We have also urged EPA to require caged trials that replicate the environments that the mosquitoes will be released into. The US Department of Agriculture required such caged trials for the GE Diamondback moth that Oxitec wanted to release in New York State. Only after the caged trials (one had to be repeated) did the USDA allow a very small open release. EPA should have required such environmentally specific caged trials for the GE OX5034 mosquito. During such trials more information on the effect of tetracycline on female survival could have been tested." (0396 p. 3)

The Center for Food Safety and GeneWatch UK stated:

> "The EPA has concluded that OX5034 'is not expected to establish within the test area' (p.94, Response to Comments). However, this conclusion seems to rely on a caged trial conducted in England to argue the transgene will not persist (p.38 to 39): this does not make sense because the caged population collapsed completely, which would not happen in the real world where wild mosquitoes are mobile and can move elsewhere. Further, in a caged trial the GE

> mosquitoes will not be exposed to tetracycline and the duration of the trial may not be sufficient to allow resistance to develop." (0383, p. 6; 0396 p. 5)

GMO Free Florida stated that:

> "Although further demonstration of efficacy would be necessary before Oxitec could submit an application to register a pesticide under section 136a of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), more laboratory and caged trials are first essential to establish that use of the pesticide under the permit, and its method of delivery via living genetically engineered (GE) pest organisms, does not cause unreasonable adverse effects on the environment." (0397 p. 38)

Friends of the Earth stated that:

> "There have not been caged trials in the proposed counties in California that show that females wouldn't survive, particularly in the presence of tetracycline. . . . There should also be caged trials in each of CA's 12 counties, and in Monroe County, FL, ahead of any open release." (0394 pp. 1 & 2)

Friends of the Earth called for:

> ". . . long term caged trials in each California county . . . ." (0394 p. 1)

Anonymous (0406) stated:

> "Why don't you test these out in a controlled environment to see what the long term risks are, like we are now discovering with GMO's? Once these are released into the world, there's no turning back." (0406 p. 1)

Craig Zabransky, questioning whether the science benefits out weight the risks, stated:

> "the local mosquito board and Oxitec skipped a recommended cage trial, a recommended step by the WHO" (0452 p.1)

Florida Keys Environmental Coalition argued against:

> " . . . the EPA's permission to skip critical safety steps, such as cage trials, prescribed by the World Health Organization (WHO) and numerous globally recognized experts practicing in this field of study in the US, prior to wild release of GM species." (0400 p. 4)

Environmental Keys Environmental Coalition further argued, with regard to "GMMs" against:

> " . . . abruptly introducing them into a wild sensitive ecosystem without taking prudent steps, like cage trials, to witness the effects positive and negative and determine if the product is effective enough to warrant further risk." (0400 p. 5)

EPA Response to Unit IX. - Comments Calling for Additional Information or Testing. With regard to comments calling for additional testing, most of the concerns expressed in these comments revolve around a perceived dearth 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 2019 request for an EUP and the 2021 request for amendment and extension of the EUP. In addition, EPA also utilized information in the published literature to assess the requests. All of this information was used to reach a determination on the requests, including, for example, to address questions on OX5034 vectorial capacity, the possibility that adult females with the OX503 traits might occur in the test areas, effects of OX5034 male releases on other animals, the potential for the OX5034 proteins to exhibit toxicity or allergenicity and effects of the use of tetracycline to produce OX5034 male mosquitoes on antibiotic resistance in microbial populations. The details of the assessment responding to the 2021 request to amend and extend the EUP, as well as the assessment for the 2019 EUP request, can be found in the Human Health and Environmental Risk Assessments, and the Reviews of Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 *Aedes aegypti* Mosquitoes. These documents can be found in the docket established for these actions (EPA-HQ-OPP-2019-0274).

EPA Response to Comments in Unit IX.A - Comments on Reproducibility of Studies by Independent Experts. With regard to requests 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. For related discussion, see EPA Response to Unit X.N.- Comments Stating that EPA Should Not Rely on the Registrant Provided Assessment Data/Information.

EPA Response to Comments in Unit IX.B - Comments on Calls for Additional Information or Testing that are Specific to the Experimental Area. Regarding the comments on introgression and vector competence, EPA received similar comments from the public on the original EUP for the OX5034 strain proposed for testing in Florida in 2020. EPA has responded to these comments in Unit V.B of the document entitled "Response to Comments to the Notice of Receipt of an Application for an Experimental Use Permit Number 93167-EUP-E" (EPA-HQ-OPP-2019-0274-0355). Additionally, as outlined in the Memorandum on Vectorial Capacity (EPA-HQ-OPP-2019-0274-0351), the EPA discussed the limited role of mosquito genetics in vector competence as well as how due to environmental factors,

viral strain variation, and mosquito population genetic variation, vector competence can vary significantly within a mosquito species both geographically and temporally, thereby limiting utility of comparative crosses. These documents can be found in the docket EPA-HQ-OPP-2019-0274 on www.regulations.gov. Because no new arguments were brought forward, the Agency refers the commenters to these previous responses.

Regarding the comments that resistance to the female-lethal trait may be observed as a result of differences in the genetic background of mosquitoes, the commenters cite an article published by Knudsen and colleagues. EPA carefully evaluated the results of this article as part of the original risk assessment (Document number: EPA-HQ-OPP-2019-0274-0359; Unit II.C.4 "Potential for resistance during field trials"). In that assessment, EPA stated that "*… genetic backgrounds have been hypothesized to play a role in resistance development through epigenetic effects (Knudsen et al. 2020)*." The review went on to conclude that "*However, penetrance of the OX5034 sex-specific lethal trait has been shown to be 100% in OX5034 homo-and hemizygous individuals of the LWT background as well as in hemizygous individuals collected from the field resulting from matings with a genetically distinct background and thus the risk from epigenetic effects is expected to be negligible*." The assessment similarly holds true for the current EUP amendment and extension.

Regarding the comment on the dispersal of OX5034 to an area where tetracyclines may be present in the environment, EPA refers the commenter to Unit IV.B for a discussion of environmental tetracycline sources.

EPA Response to Comments in Unit IX.C - Comments Calling for Additional Information or Testing for Toxicity or Allergenicity of OX5034 Transgenic Proteins. With regard to the comments that the EPA requires no safety studies in its approval process for testing of OX5034 under an EUP, has set no standards for success or failure of this experiment, and requires no reporting of data, the commenters are incorrect. EPA's 2020 and 2022 Human Health and Environmental Risk Assessments are based upon a careful analysis of data and other information required by EPA and supplied by Oxitec. These documents can be found in docket EPA-HQ-OPP-2019-0274.

Regarding the comments calling for additional testing of the tTAV-OX5034 and DsRed2-OX5034 proteins to determine their potential for being allergenic or toxic, as noted in Unit V.C, the risk that a pesticide poses to human health is a function of hazard and exposure. As discussed in Unit II.B.3.b of the Human Health and Environmental Risk Assessment for the original EUP for OX5034 (Docket number: EPA-HQ-OPP-2019-0274-0359), the most relevant exposure to tTAV-OX5034 and DsRed2-OX5034 would be expected from biting OX5034 females *if* they were present in the environment. However, 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 in the context of the original EUP as well as the current EUP amendment and extension.

Regarding the comment suggesting specific rodent and non-rodent studies to determine the potential for toxicity, EPA refers the commenters to the biochemical and microbial data requirements listed in 40 CFR part 158, which are used to register biopesticides, and have aided EPA in determining appropriate data needed for the Agency to evaluate a modified mosquito product intended for open field testing under an EUP. Among those are specific requirements for determining the potential for adverse effects through the oral route of exposure. In general, a data requirement identified as appropriate for a modified mosquito product can be met through generating the data identified in the data requirement or, in lieu of generating such data, by submitting or citing results from previously conducted studies, and/or citing publicly available literature. As discussed in Unit V.B. of this Response to Comments document (EPA-HQ-OP2019-0274-0355) and Table 4 of the risk assessment for the original OX5034 EUP (docket number: EPA-HQ-OPP-2019-0274-0359), EPA carefully evaluated the likelihood of oral exposure and found oral exposure to OX5034 mosquitoes to be negligible. The overall risk assessment formula specifies that risk = hazard x exposure. Thus, while EPA did not make a determination on the oral toxicity for the original 2020 EUP, because oral exposure was determined to be negligible, the overall risk of OX5034 was determined to be negligible. That risk conclusion did not change for the current amendment and extension request.

Regarding the comment requesting that tests be done to determine the levels of known allergenic proteins in the saliva of OX5034 females, as discussed in the paragraph directly above (and citations therein), only male OX5034 will be released into the environment, and male mosquitoes do not pose a biting risk to humans. Regarding the comment that the mutation rate may lead to the presence of female OX5034 in the environment, EPA refers the commenter to its response to Unit IV.C. – Comments on the potential emergence of resistance to arise to the OX50304 trait – that discusses this question in detail.

EPA Response to Comments in Unit IX.D - Comments on Calls for Additional Testing or Information on Environmental Considerations. With regard to the comments requesting testing on specific taxa, testing on organisms local to the test sites, and long term (e.g., multigenerational) testing, EPA refers to the biochemical and microbial data requirements listed at 40 CFR part 158, which are used to register a biopesticide, and have aided EPA in determining appropriate data needs for a modified mosquito product. Data identified as needed for a modified mosquito product can be met through generating the data identified, including through the use of alternative species, or in lieu of generating such data by submitting or citing results from previously conducted studies, and/or citing publicly available literature. Data needed address both components of a risk assessment, i.e., the potential for hazard that the pesticide presents, and the estimated level of exposure to humans or nontarget species, including the potential for gene flow and dispersal. EPA carefully examined the data and information submitted to the Agency in support of the 2019 request for an EUP and the 2021 request for amendment and extension of the EUP. In addition, EPA also utilized information in the published literature to assess the requests. All of this information was used to reach a determination on the requests, including to address for example, questions on the potential for the OX5034 proteins to exhibit toxicity and effects of OX5034 male mosquito release on the ecosystem. The details of the assessment responding to the 2021 request

to amend and extend the EUP, as well as the assessment for the 2019 EUP request, can be found in the Human Health and Environmental Risk Assessments. These documents can be found in the docket established for these actions (EPA-HQ-OPP-2019-0274).

With regard to the comment on Spain's National Biosafety Commission request to Oxitec that predator studies be done for another of Oxitec's products, EPA declines commenters' invitation to determine obligations Oxitec may have to Spain's National Biosafety Commission. Whatever such obligations may be, they would be owed by Oxitec to Spain; EPA must determine Oxitec's legal obligations in the United States under FIFRA. Such obligations will be determined by the dictates of FIFRA and the characteristics of the pesticide.

EPA Response to Comments in Unit IX.E - Comments on Testing and Antibiotic Resistance. With regard to the comments requesting testing of OX5034 mosquitoes for antibiotic resistant bacteria, EPA's analysis of the likelihood that OX5034 mosquito could carry tetracycline resistant bacteria can be found in the 2020 Response to Comment document. Please see section "EPA Response to Unit VIII.C. – Comments on Potential for Environmental Releases of OX5034 to Contribute to Increases in Antibiotic Resistance in Microbial Populations" of the Response to Comment document associated with the 2020 EUP (EPA-HQ-OPP-2019-0274-0355).

EPA Response to Comments in Unit IX.F - Comments on Calls for Caged Trials Prior to Open Field Testing. With regard to comments stating that EPA should require caged trials for OX5034 prior to permitting EUP testing, based on its assessment, EPA does not find it necessary to require caged trials for OX5034 prior to EUP testing given the type of organism being tested and the limitations placed on the field trial. This position is consistent with the guidance offered by the document to which commenters refer, the World Health Organizations (WHO) "Guidance Document for Testing Genetically Modified Mosquitoes,"[27] which states that the decision on the appropriate testing conditions:

> " . . . will be made by national regulatory authorities and will probably depend on the nature of the GMM technology, prior knowledge of its effects in other environments and other factors that are taken into account in the RA process . . . a physically confined trial might not be deemed necessary, for example, when a technology has already been tested and found to be safe in another venue." (p. 14)

In addition, it should be noted that OX5034 mosquito falls into the category that the WHO guidance describes as "self-limiting and localizing" because the female progeny of the released male mosquitoes do not survive to emerge as adults. WHO guidance states that for this type of Genetically Modified Mosquito (GMM):

> "The modification will be passed on according to Mendelian inheritance, becoming less prevalent within the population over successive generations of crossing with native mosquitoes.

[27] http://apps.who.int/iris/bitstream/handle/10665/341370/9789240025233-eng.pdf?sequence=1&isAllowed=y

> The fitness cost associated with the modification will accelerate the loss of this type of GMM from the population." (pp. 8-9)

X. Comments Recommending Actions EPA Should Take

Comments were received recommending actions that the EPA should take with regard to the request to amend and extend the EUP testing of the Oxitec OX5034 mosquito. Most of these comments revolve around: (1) Requests that EPA extend the comment period; (2) requests that the Agency be transparent in its decision making; (3) the public's desire for an opportunity to be part of the decision making process, including ensuring that the public has an opportunity to review the data supporting the request; (4) the public's desire that the Agency ensures that scientific expertise external to the EPA has an opportunity to review the data supporting the request; (5) requests that the EPA put in place a mitigation strategy to use in the event that the OX5034 mosquito does not behave as anticipated, and (6) concern about potential economic consequences from the testing of OX5034 mosquito.

A. Comments Requesting That EPA Extend the Comment Period

Ten comments (0382, 0387, 0397, 0400, 0405, 0411, 0440, 0451, 0460, 0461) requested an extension of the comment period allotted for the Notice of Receipt for the Oxitec application requesting an amendment and extension to the existing EUP. The amendment and extension of the EUP would allow Oxitec to continue to evaluate the efficacy of releasing male OX5034 mosquitoes as a tool to suppress wild *Ae. aegypti* mosquito populations.

GMO Free Florida requested:

> ". . . an extension to the comment period as 30 days is not enough time to evaluate the risk for all species, including endangered species, in the state of California. This is necessary as the proposed locations in California have not been mentioned. In fact, information on this proposed experiment for California is completely lacking altogether. Therefore, it is necessary for the EPA to provide the necessary information and extend the duration of the comment period so that the public has the ability to properly comment on this issue." (0387, 0397 p. 1)

Florida Keys Environmental Coalition also entered a request for "EUP Comment Period Extension" and argued that:

> " . . . there is significant value for all to extend the comment period by 60 days and actively inviting expert input to improve the quality of commentary and the breadth of thought." (0400 p. 3)

The Center for Food Safety requested a 60 day extension, stating that:

> "When I talked with EPA staff after the docket was first opened, I was only told that 12 counties in California were being considered for the release of the Oxitec mosquitoes. Since then (on September 14) 10 mosquito control districts in California have posted to the docket that they want to have the mosquitoes released in their districts. These districts represent at least 11 counties (Alameda, Riverside, Fresno, Tulare, Stanislaus, Angeles, Orange, Sacramento, Yolo, Shasta and San Bernardino). We would like to have more time to look at the environmental and human issues raised by the release of the mosquitoes across such a wide ecological area. The EPA docket does not provide this information. Such information and information on the demographics of the counties would help our comments. We believe that a 60 day extension would allow us time to do that research." (0382 p. 1)

The Institute for Responsible Technology stated that:

> " . . . we also request a 60-day extension of the comment period so the citizens of the unnamed California counties where the gene altered mosquitoes are targeted can be fully informed and have an opportunity to educate themselves and make meaningful comments to the EPA." (0461 p. 1)

Michael Burton (0440) stated that:

> "Please provide a 60 day extension so we can get a better picture of the plans and risks of such a potentially disastrous release." (0440 p. 1)

Bobbie Flowers, Penelope McMillan, Bruce Campbell and Comment 0460 representing Mass Mailer requested that EPA:

> " . . . extend the public comment period for this application." (0405p. 1; 0411 p. 1; 0451 p. 1; 0460 p.1)

B. EPA Should Ensure Transparency

Some commenters (0383, 0388, 0394, 0396, 0433, 0399, 0400, 0401, 0405, 0410, 0412, 0418, 0426, 0430, 0433, 0434, 0436, 0441, 0452, 0460, 0461) raised concerns that revolve around transparency of the Agency in its actions, including transparency in the materials the Agency included in the docket. Some of these commenters requested that the Agency reveal all of its analyses of the OX5034 proposal, or in other ways provide more information to the public. Some comments specifically addressed CBI claims, pointing out how EPA acceptance of the claims limited the public's ability to comment; some of these comments identified topics of particular concern that had been claimed as CBI. Others argued that EPA should challenge or otherwise not allow CBI claims. Others simply stated that Oxitec does not share its data with the public, and thus did not allow the public to participate in discussing issues of importance to the public.

1. Comments arguing in general for greater transparency

Comments in this category (0388, 0394, 0396, 0399, 0400, 0410, 0412, 0426, 0430, 0433, 0434, 0436, 0441) complained of a lack of publicly available information.

Friends of the Earth stated that:

> "As with Oxitec's 2018 EUP application for releases in Florida, the lack of transparency and missing information makes any meaningful independent assessment nearly impossible." (0394 p. 6)

The Center for Food Safety stated that:

> "Like the original application, no risk assessment and no review of the environmental or human health effects are provided for the public to review." (0396 p. 1)

Judith Borcz, Andria Ventura and Deborah Ramelli stated that:

> "Regarding the Florida proposal to extend the field trials, there has been no data given to the public from the ongoing field trials, giving the public nothing to assess." (0430 p.1; 0434 p. 2; 0436 p. 1)

Christopher Lish stated that:

> "Neither the Environmental Protection Agency nor Oxitec have provided sufficient data from the ongoing field trial, peer-reviewed or otherwise, on which to base any substantial public comment, giving the public nothing to assess." (0388 p.1)

K. Bluefield stated that:

> "Like the last application to the EPA, there is no current data presented in this proposal on which the public should be able to offer an assessment and useful comments." (0433 p. 1)

Pamm Larry stated that:

> "And now Oxitec wants to expand its experiment and release these genetically engineered insects without providing sufficient data on which we can, as a democratic society, make an informed decision on the course of the planet and our children's future." (0441 p. 1)

Friends of the Earth stated that:

> "The current information available to the public for review is inadequate and blocks critical information necessary for responsible analysis of environmental and public health risks. In addition, the public engagement process as witnessed in Monroe County, Florida has lacked transparency, been riddled with contradictions, and misled the public." (0394 p. 6)

Florida Keys Environmental Coalition stated that:

> "The 2019 - 20 application and approval of this technology by the EPA was an obfuscated internal and interagency evaluation, with irrationally short comment period and insufficient information being made available from the vendor, only to have what technical information that has been made available emerge immediately after the approval." (0400 p. 4)

Julie Ostoich and Karen Barranco stated that:

> "We don't know the long term impacts of genetically engineered mosquitoes. Oxitec does not disclose their data." (0410 p. 1; 0412 p. 1)

Kimberly Sikora stated that:

> "As residents we are not offered any results by Oxitec, and as such, the results remain unknown. . . . I have attended numerous webinars and zoom meetings by FKMCD & Oxitec and my questions and concerns have never been addressed or answered. The information they answer and provide is cherry picked and they only respond to the questions that put a favorable spin on Oxitec." (0426 p. 1)

Meagan Morrison Hull stated:

> "Oxitec created a contract signed by Florida Keys Mosquito Control (FKMC), which stipulates that every aspect of this experiment is proprietary. Furthermore, no data, no results, and no information whatsoever, may be disclosed." (0399 p. 2)

2. <u>Transparency and Confidential Business Information (CBI)</u>

Several comments (0383, 0394, 0396, 0400, 0401, 0461) addressed how CBI affected the ability of the public to participate. Some comments (0400) offered opinions on how EPA should treat CBI.

Florida Keys Environmental Coalition argued that:

> "Confidential Business Information CBI should be carefully qualified as appropriate for redacting or withholding, and bias should be afforded to the public in cases where human and ecosystem

> experimentation is proposed. Not requiring full disclosure especially where GM Species are being deployed is negligence. Businesses have a choice when seeking to achieve regulatory approval in the US and sufficient transparency must be provided to our citizens, or products should be considered unacceptable. This requirement is not unusual, onerous, or damaging to Oxitec, given that most businesses would not purchase a product or permit an experiment with a product if a vendor withheld concerning performance or safety data." (0400 p. 3)

Florida Keys Environmental Coalition further argued that:

> "This vendor should not be permitted to withhold information that in the past was not considered "Confidential", such as lab data that supports, or disputes claims made in the application. It was this very data that permitted clear understanding that introgression would occur with the OX513A and that there was a significant functional flaw in the leaky nature of the lethal gene's performance, opening a likely failure mode. This failure mode together with an inept sex sorting capability was clearly demonstrated in the failed Cayman experiment from 2017 -18. We would argue that this flaw, manifested as a broad variation of female and male survivability of approximately 2 days to in excess of 50 days, was understandable because of the laboratory data provided." (0400 p. 3)

Florida Keys Environmental Coalition argued that:

> "The EPA permitted Oxitec to withhold all technical data prior to approval and then released all data post approval. When queried about this, the EPA claim was that Oxitec withheld and did not permit release until then. Perhaps the EPA should have challenged the process at that point," (0400 p. 2)

The Center for Food Safety stated that:

> "After EPA approved the original trials in Florida and Texas, EPA posted to the docket nine (9) documents, including a risk assessment. Despite my having asked the EPA staff to post to the docket any materials that they have that would answer these questions for the California trials and any data on the Florida releases to date, the EPA staff insisted that this was all confidential business information of Oxitec. In essence, EPA is admitting that what should be a public review of a new technology is a SECRET discussion between EPA staff and Oxitec staff. The staff moreover told me and a colleague from another public interest group that we should be happy that they were having a docket for public comments at all as this in not legally required." (0396 p. 1) [Emphasis in Original]

The Center for Food Safety further noted that:

> " . . . the risk assessment (EPA-HQ-OPP-2019-0274-0359) of the Florida/Texas trials were not made public for the earlier consultation. Even when it was released on May 28, 2020 key sections were claimed by Oxitec as Confidential Business Information and redacted: i.e. page 18 (mosquito rearing); page 21 (fecundity); page 22 (longevity) and, most troubling, a long section on p.28 which is the discussion of the allergenicity of the genetically engineered fluorescent protein inserted to track the mosquitoes (DsRed2-OX5034). Not redacted, but nonetheless inadequate, is the discussion of the amount of tetracycline exposure needed for female OX5034 mosquitoes to survive to mate and produce offspring. Given that both the environments of California contain many sources of tetracycline, this is an important fact to know. The risk assessment only points to secret Oxitec documents that the public is not given access to in the risk assessment and have not been published and peer-reviewed." (0396 p. 2)

The Center for Food Safety stated that:

> "The EPA can only restore public trust in its judgements if it starts making all of its decision making fully transparent. If companies need legal protections for their inventions, that is what the US Patent and Trademark Office provides. EPA should get out of the business of making behind closed doors deals with biopesticide companies like Oxitec." (0396 p. 9)

Florida Keys Environmental Coalition argued that:

> ". . . CBI is intended to be reserved for that proprietary information that may disclose trade secrets, not because the vendor desires to disadvantage the people they want to experiment on from making relevant comments." (0400 p. 4)

Florida Keys Environmental Coalition argued that they see no:

> " . . ., validity for the designation of non-proprietary CBI and request the EPA justify the type of information being withheld . . . ." (0400 p. 5)

Californians for Pesticide Reform stated that "[I]nadequate information assessing the health and safety to people and the environment" had been provided:

- "It's also impossible to understand the full risks when some sections of the risk assessment (EPA-HQ-OPP-2019-0274-0359) remain blacked out as Confidential Business Information (CBI), most concerningly 15 lines on p. 28 about the allergic potential of the fluorescent protein DsRed2-OX5034. The section on tetracycline in the environment (p.32) does not say what levels are required for female OX5034 mosquitoes to survive to adulthood. The risk assessment also refers to numerous Oxitec studies which are not public.
- The EPA's 'Response to Comments' (p.132) also refers to CDC advice: however, the full advice has not been provided.

- There is no published information regarding Oxitec's earlier releases of OX5034 GE mosquitoes in Brazil." (0401 p.2)

Friends of the Earth stated that:

> "Information critical for health and environmental analysis is blacked out and withdrawn as Confidential Business Information (CBI). Public health information withdrawn or redacted from the application includes: details about the allergic potential of the fluorescent protein found in the mosquito's saliva, and details about what levels of tetratcycline [sic] would allow female GE mosquitoes to survive to adulthood. Given the human populations in California and Florida, this public health information should not be allowed to be withdrawn as CBI. The EPA has also not included the CDC's full advice." (0394 p. 6)

GeneWatch UK noted that the "environmental risk assessment and other documents were not published until the earlier consultation (based on only 2 pages of information from Oxitec)" for the original EUP permit issued in April 2020 was complete and that these raise "additional questions and concerns," specifically:

- "Some sections of the risk assessment (EPA-HQ-OPP-2019-0274-0359) remain blacked out as Confidential Business Information (CBI): one line on p. 18 (mosquito rearing); one line on p.21 (fecundity); 2 lines on p.22 (longevity); 15 lines on p. 28 (about the allergic potential of the fluorescent protein DsRed2-OX5034). The section on tetracycline in the environment (p.32) does not say what levels are required for female OX5034 mosquitoes to survive to adulthood. The risk assessment also refers to numerous Oxitec studies which are not public.
- "The EPA's 'Response to Comments' (p.132) also refers to CDC advice: however, the full advice has not been provided . . . .
- "No information regarding populations of *Aedes aegypti* in California has been provided, nor any evidence regarding competitor species or predators/prey. This makes it impossible to assess environmental risks.
- "There is also no published information regarding Oxitec's earlier releases of OX5034 GE mosquitoes in Brazil." (0383 p. 1 and 3)

The Institute for Responsible Technology argued that "EPA Assessments Are Biased To Favor Industry" when:

> "By labeling data "confidential business information," Oxitec conceals relevant test results from the public. (0461 p. 2)

3. <u>Specific information commenters believe should be provided to the public</u>

Commenters in this category (0380, 0388, 0394, 0396, 0397, 0401, 0405, 0412, 0408, 0418, 0430, 0434, 0436, 0441, 0461) identify information they believe should be available to the public. This includes: (1) a listing of the 12 California counties in which Oxitec is proposing a release, (2) results of trials with Wolbachia infected mosquitoes, (3) full results of the Oxitec trials in Florida and Brazil, and (4) details on containment and labeling.

Judith Borcz, Andria Ventura and Deborah Ramelli stated that:

> "The docket did not list the 12 California counties in which Oxitec is proposing a release, nor does it discuss the potential impacts on unique ecosystems and endangered species habitat in California. The docket does not discuss the significant agricultural lands in California and the associated chemicals used in these settings, such as tetracycline, that would impact any field trial. Without sufficient and publicly available science, data, analysis, or identification of need, we do not support the field trial proposal for California." (0430 p. 1; 0434 p. 1; 0436 p. 1)

Karen Barranco stated that:

> "We demand transparency as to which countries in California are being targeted for this experiment and for what purpose it is being used." (0412 p. 1)

Institute for Responsible Technology stated that:

> " . . , EPA is withholding the list of 12 counties being considered for the GE mosquito release. This prevents reviewers from analyzing relevant data about counties' ecosystems, endangered species, use of tetracycline in agriculture (see below), and prevalence of the mosquito-borne diseases that the GE insects are designed to reduce." (0461 p. 3)

Californians for Pesticide Reform stated that:

> "EPA and Oxitec did not provide sufficient data, peer-reviewed or otherwise, on which to base any substantial public comment. The docket did not list the 12 California counties in which Oxitec is proposing a release, . . ." (0401 p. 1)

Pamm Larry stated that:

> "I request that Oxitec and the EPA specify where the 12 proposed California counties are. I request that there be assessments in each county before moving forward, as each county is different with different considerations. We have a number of species on the endangered list in CA and the impact of this experiment should not be overlooked in regards to protecting them from potential long-term impacts on our bioregion and all species in it." (0441 p.1-2)

The Center for Food Safety stated that:

> "The Center for Food Safety noted in its review of the original proposed release of Oxitec OX5034 GE mosquitoes that inadequate information was being provided to review proposed releases in Florida and Texas. That application, ironically, contained more specifics than does the current application. At least the original application indicated which counties in Florida and Texas would have mosquito releases. The two page extension to the application does not indicate which California counties would take the mosquitoes. A letter from 10 mosquito control districts posted to this docket suggests that the 11 counties where those mosquito control districts have operations are the likely sites of the releases." (0396 p. 1)

Regarding information on trials for a different modified mosquito, *Wolbachia* infected mosquitoes, Judith Borcz, Andria Ventura and Deborah Ramelli stated that:

> "Without data about the genetically engineered Aedes agypti [sic] mosquito trails [sic], as well as other field trials from Wolbacchia infected mosquitoes, the public is not able to understand the impacts of this release." (0430 p. 1; 0434 p. 2; 0436 p. 1)

Some commenters requested information on Oxitec's previous trials. The Center for Food Safety contended that EPA should not proceed with these trials until it:

> "Releases in a timely manner all data from trials already conducted with GE insects and related trials such as those involving Wolbachia infected mosquitoes." (0396 p. 10)

Friends of the Earth stated that:

> "There is also missing information critical for environmental assessments. Neither EPA nor Oxitec publicly name the counties proposed for release. There is no information about populations of Aedes aegypti in California or competitor mosquito species that could move into its ecological niche, and it remains unclear how any analysis about population reduction will be conducted. Despite Oxitec's previous completed field trials in Brazil and Florida, there is still no publicly available data." (0394 p. 6)

Penelope McMillan stated that:

> "EPA must collect all available data from Oxitec's experiment in the Florida Keys and release it to the public for review." (0405 p. 1)

Jerry Russell stated that:

> "Oxitech (sic) should be required to release full results of their trial in Florida before expanding to other areas." (0418 p. 1)

The Institute for Responsible Technology stated that:

> "EPA is asking for public comments and preparing its own decisions on whether to extend the Florida trial for two years and start other trials in California without ever making the results of the current mosquito trial available." (0461 p. 2)

Christopher Lish stated that:

> "Regarding the Florida proposal to extend the field trials, there has been no data given to the public from the ongoing field trials, giving the public nothing to assess. Without data about the genetically engineered *Aedes agypti* mosquito trails, as well as other field trials from Wolbacchia infected mosquitoes, the public is not able to understand the impacts of this release." (0388 p. 1)

GMO Free Florida, arguing that some considerations applied to Oxitec's OX513A would also apply to OX5034, contended that information on OX513A should also be provided to the public:

> ". . . Oxitec . . . documents provided to the FDA and "SECTION G PROPOSED EXPERIMENTAL PROGRAM" along with other data provided to the EPA: The FDA documents are specifically about OX51A, however, the OX5034 mosquito carries many of the key features of OX513A." (0397 p. 1) [Footnotes Omitted] [Emphasis in Original]

Frances Micklam expressed concern that there was no:

> " . . . discussion of how GM insects can be contained at a site, or products produced using GM insects can be labelled;" (0380 p. 1)

Maggie Mistal stated that:

> ". . ., I have not been happy with how the tests have been conducted here. We have not been told where the boxes are with the GMO mosquitos. We don't know if and how our environment is being impacted." (0408 p. 1)

C. Comments Arguing That EPA Should Seek Advice from Independent Experts

Sixteen comments (0372, 0383, 0388, 0394, 0396, 0400, 0401, 0405, 0408, 0430, 0433, 0434, 0436, 0452, 0460, 0461) argued that EPA should seek advice on the OX5034 mosquito from independent experts. Two of these commenters (0396, 0400) specifically requested EPA convene a FIFRA Scientific

Advisory Panel (SAP). Seven (0383, 0388, 0397, 0401, 0430, 0433, 0434, 0436) were more general in their request that EPA involve experts in the reviews.

1. Requests that EPA convene a Scientific Advisory Panel (SAP)

Comments in this category (0396, 0400) specifically requested that the EPA obtain advice from a group of independent experts by convening the FIFRA Scientific Advisory Panel (SAP).

The Center for Food Safety stated that:

> "EPA should not proceed with these trials until it: Convenes a Scientific Review Panel composed of multidisciplinary specialists to independently review these genetically engineered "biopesticides". (0396 p.9)

The Center for Food Safety further stated that:

> "We have urged the EPA to convene a Scientific Advisory Panel to review this new "pesticide" as it has with many other new pesticides, i.e. Nanosilver as a pesticide. Yet the EPA did not convene an independent, external scientific advisory panel to review Oxitec's claim; the agency's risk assessment was only made publicly available after their approval decision and we know of no peer-reviewed articles on this particular GM mosquito strain." (0396 p.3)

The Center for Food Safety went on to state that:

> " . . . an external independent group of experts (A scientific review panel) should be convened to review the first GE mosquitoes presented for release. But to address the complexity of such a decision, this group should consist of interdisciplinary experts representing diverse identities with expertise in ecology, genetics, vector biology, risk assessment, entomology, public health, ethics, and social science. External peer review is a cornerstone of good science and could ensure that all necessary risks are being addressed." (0396 p. 2)

The Florida Keys Environmental Coalition stated that:

> "We are asking for an immediate suspension of the current EUP until a Scientific Advisory Panels can be convened to define a proper objective independent scientific evaluation process and administer the execution of that process through fruition for the OX5034 GMM. Our arguments question the EPA performance and divergence from precedent in granting the original EUP approval, where EPA stated standards and recommended practices were not applied." (0400 p. 1)

The Florida Keys Environmental Coalition stated that:

> "A vast array of qualified objective scientists are available within the US that could assist in establishing interim protocol and standards for evaluating and measuring the short and long term risk and performance concerns, when measured against the back drop of variables that exist within subspecies and wild ecosystems. Instead, the EPA's lack of a defined process, or standard has resulted in very concerning outcomes with a less than proficient understanding of the technology created by the vendor." (0400 p. 5)

The Florida Keys Environmental Coalition further stated that:

> "These challenges will only heighten as other genetic engineering and modification methods mature. Zinc Finger, CRISPR-Cas9 and a myriad of other techniques are already in practice and the basis of numerous developments on the horizon. We urge the EPA of the importance of embracing regimented adaptive evaluation protocols via a programmatic practice of routinely engaging Scientific Advisory Panels (SAPs) to assure more comprehensive consideration of each product each . . . .as knowledge is gained." (0400 p. 5)

The Florida Keys Environmental Coalition added that:

> "We ask the EPA to use this opportunity with the considerable expansion, the broad and varied outcomes possible due to the vast array of venues proposed, to convene a Scientific Advisory Panel with the intension of architecting and comprehensive independent scientific investigation of this product and that any prescribe, or permitted testing follow prudent steps, such as cage trials and results analysis prior to any open field releases of the OX5034. We believe the only good technology is that based in quality scientific programs and practices that can best be administered with independent objective peer review and that this process yields benefits for all stakeholders and a pathway to change needed with our regulatory agencies as the rate of scientific challenge accelerates." (0400 p. 10)

The Florida Keys Environmental Coalition stated that the EPA "is remised and negligent" by not:

> ". . . [U]tilizing the benefit of a Scientific Advisory Panel and seeking to perform and Environmental Impact Statement to assure the technology is well understood and vetted by independent qualified scientific processes, not a vendor claim submission." (0400 p. 9)

Florida Keys Environmental Coalition questioned EPA's expertise and argued industry bias stating that:

> "Evidence of the EPA's complicity with the vendor and questionable internal expertise to properly administer an evaluation without appealing to a Scientific Advisor Panel (SAP) for this technology is seen in specific action the EPA has taken and displayed. . . . Divergence from

> precedent in failing to call together a SAP when vendor research claims are all that is provided for proof of performance and risk analysis." (0400 p. 2)

2. General comments that EPA should involve independent experts in reviews

Comments in this category (0372, 0376, 0383, 0388, 0397, 0394, 0396, 0397, 0400, 0401, 0408, 0430, 0433, 0434, 0436, 0441, 0460, 0341) argued that transparent independent scientific review should be a part of EPA's approach to OX5034 and other genetically engineered products, but did not specify the mechanism through which such review should occur.

GeneWatch UK urged that EPA:

> "Establish an independent 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 and consider the potential environmental, health and social impacts of the release of GE insects" (0383 p. 7)

Californians for Pesticide Reform argued that "[W]hile limiting mosquito populations and the spread of mosquito borne disease is important, this experiment has still not undergone any transparent independent scientific review, which should be a requirement," EPA should not "approve this application, nor any future releases of GE mosquitos, until and unless:"

- "An independent expert committee has been established to produce a publicly available, peer-reviewed analysis of environmental, health and social impacts of any genetically engineered insect release proposal;"
- "The committee of independent ecologists and entomologists, public health experts (including dengue fever and zika virus specialists), and other key experts and public stakeholders have reviewed the proposal". (0401 p. 3)

Christopher Lish, Judith Borcz, K. Bluefield, Andria Ventura and Deborah Ramelli stated that "[W]hile limiting mosquito populations and the spread of mosquito borne disease is important, this experiment has still not undergone any transparent independent scientific review, which should be a requirement," thus EPA should reject Oxitec's request and:

- "Establish an independent committee to produce a publicly available peer-reviewed analysis of the environmental, health and social impacts of the genetically engineered insect release proposal;"
- "Have the independent 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 and related data . . . " (0388 pp. 2-3; 0430 p. 2; 0433 p. 2; 0434 p. 2; 0436 p. 2)

Florida Keys Environmental Coalition argued that:

> "The regulatory process needs to change and adapt to the accelerating rate of novel technology development. It is for the EPA to manage, but reliance on independent expert scientist should be the norm, not the exception." (0400 p. 7)

Florida Keys Environmental Coalition stated that:

> "Standard practices and EPA precedent if followed would require the Oxitec performance and risk analysis claims submitted should be subjected to objective independent qualified scientific investigation to determine the actual performance and risk prior to subjecting the US public, or ecosystems to harm." (0400 p. 2)

Californians for Pesticide Reform stated that:

> "We are particularly concerned about the proposed expansion of the company's experimental releases of GE mosquitoes to the state of California without adequate peer-reviewed analysis of potential health and environmental impacts." (0401 p. 1)

GMO Free Florida urged that "EPA must reject Oxitec's application for genetically engineered mosquitoes and":

> "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." (0397 p. 38)

Friends of the Earth stated that EPA should produce a EIS for each of the counties proposed as test sites:

> ". . . and this should be reviewed by a committee of independent ecologists and entomologists, public health experts, and other key experts and public stakeholders." (0394 p. 1)

Center for Food Safety stated that "EPA should not proceed with these trials until it:

> "Develops a program of independent review of all aspects of the trials, including the sterile insect strategies being used." (0396 p. 9)

Penelope McMillan and Comment 0460 representing Mass Mailer expressed concern that:

> "Potential human health impacts have not been researched by any third party." (0405 p. 1; 0460 p. 2)

Kathryn Woodfine stated that:

> "We need a truly independent scientific investigation into the Oxitec GMM technology, before any further experimentation is permitted!" (0376 p. 1)

Pamm Larry stated that:

> "EPA needs to establish an independent committee or task force comprised of a number of different scientific disciplines, public stakeholders and sectors to produce publicly disclosed, peer reviewed data on the long term impacts of release into the environment." (0441 p.2)

Maggie Mistal stated that:

> "I encourage the EPA, local officials and residents in California to get the perspective of independent scientists about the effectiveness and potential downsides of this technology BEFORE moving forward and to look seriously at other options. This is not the only way to solve the issue of mosquito born disease." (0408 p. 1) [Emphasis in Original]

The Institute for Responsible Technology stated:

> "Without independent, in-depth assessments from a variety of experts, the EPA's approval process remains a façade." (0461 p. 4)

Edward Russo expressed concern that:

> " . . . all of the findings come from Oxitec. There has been no independent analysis that confirms Oxitec's claims." (0372 p. 1)

D. Comments Arguing that the Agency Should Seek Public Input

Fifteen comments (0378, 0380, 0383, 0388, 0394, 0396, 0397, 0400, 0401, 0430, 0433, 0434, 0436, 0441, 0452) requested that the Agency seek public input into the decision-making process. Some comments (0383, 0388, 0394, 0396, 0397, 0401, 0430, 0433, 0434, 0436) requested that EPA convene meetings with the public, others (0380, 0383, 0388, 0401, 0430, 0433, 0434, 0436, 0452) argued that a referendum should be held. Some comments (0378, 0400, 0441) simply argued that the public should be involved in any decision making.

1. <u>Convene public meetings</u>

Some comments (0383, 0388, 0394, 0396, 0397, 0401, 0430, 0433, 0434, 0436) argued that EPA should convene meetings with the public to obtain input on the OX5034 proposal.

GMO Free Florida stated that once "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," EPA should:

> "Convene a public meeting in the Florida Keys, advertised in the Federal Register for the review of the company's proposal with the above committee present." (0397 p. 38)

GeneWatch UK stated that EPA should:

> "Convene public meetings, at various times of the day and evening, across all potentially affected communities for public comment and discussion of the proposal with key independent experts present;" (0383 p. 7)

Californians for Pesticide Reform argued that EPA should not "approve this application, nor any future releases of GE mosquitos, until and unless":

> "The EPA has convened public meetings, in all relevant languages, at various times of the day and evening, across all potentially-affected communities for public comment and discussion of the proposal with the . . . committee [of experts] present;" (0401 p. 4)

Christopher Lish, Judith Borcz, K. Bluefield, Andria Ventura and Deborah Ramelli stated that EPA, with a committee of experts present, should:

> "Convene public meetings, at various times of the day and evening, across all potentially affected communities for public comment and discussion of the proposal . . . ;" (0388 p. 2; 0430 p. 2; 0433 p. 2; 0434 p. 3; 0436 p. 2)

Friends of the Earth stated that:

> "EPA should also convene public meetings in Monroe County, FL and in each of the 12 California counties, advertised in the Federal Register, for the review of the company's proposal and EIS." (0394 p. 1)

The Center for Food Safety contended that EPA should not proceed with these trials until it:

> "Holds public hearings in each of the counties proposed for the release of the genetically engineered insects." (0396 p. 10)

The Center for Food Safety contended that:

> "Most important, people who live in areas of release must be consulted for their specialized, on-the-ground knowledge and for their right to have input in decisions that will affect them. In California, State and local agencies must do their own review of these OX5034 mosquitoes and decide based on public review whether to allow mosquito control districts to conduct the experiments. An independent group should host public conversations through local community venues, and it must make sure that structurally marginalized perspectives are at the center of those gatherings. But local community input should be consulted at every stage of the regulatory process, before not after permits have already been granted. And earmarked government funding from mosquito control districts, county boards, state agencies and EPA should support these local deliberations, as well as measures to amplify underrepresented people concerned about environmental regulation, biotechnology and human health." (0396 p. 4-5)

Friends of the Earth stated that:

> "EPA has not provided potentially affected communities with critical notice about the application. Community members across all 12 counties must be informed of this proposal and amendment, be informed about the public comment period, and have the full information to do assessments related to their communities. However, the counties proposed for release sites in California haven't been formally named, so people will not know if they could be impacted by the proposed release." (0394 p. 6)

Florida Keys Environmental Coalition stated that:

> "Given the novelty the technology and the breadth of the proposed regions in California, the public should be entitled to engagement with EPA representatives, meeting with the public at each venue targeted for experimentation. The public has the right to understand the risks of this technology and the performance failures of this vendor prior to any releases. Given the EPA position of advocacy for this foreign national vendor, the Agency should take responsibility for justifying approval and the lack of oversight practiced for this experimentation. There will be many questions to address in resolving the public's technical, societal, and ethical concerns." (0400 p. 4)

Florida Keys Environmental Coalition also argued:

> "The EPA zoom meeting held nearly a year after EUP approval that target at the FL Keys public and simply recited Oxitec's talking points and their generalized marketing presentation." (0400 p. 2)

2. Hold a referendum

Nine comments (0380, 0383, 0388, 0401, 0430, 0433, 0434, 0436, 0452) requested that a referendum be held for residents in the areas designated as testing areas.

GeneWatch UK stated that EPA should:

> "Conduct a referendum for Florida and California residents to vote on whether there should be a release of Oxitec's genetically engineered mosquitoes." (0383 p. 7)

Californians for Pesticide Reform argued that EPA should not "approve this application, nor any future releases of GE mosquitos, until and unless:"

> "A referendum has been conducted, so potentially-affected residents can vote on whether there should be a release of genetically engineered mosquitoes in their area." (0401 p. 4)

Christopher Lish, Judith Borcz, K. Bluefield, Andria Ventura, and Deborah Ramelli stated that EPA should:

> "Conduct a referendum for Florida and California residents to vote on whether there should be a release of Oxitec's genetically engineered mosquitoes." (0388 p. 2; 0430 p. 2; 0433 p.2; 0434 p. 3; 0436 p. 2)

Frances Micklem, concerned about possible negative consequences of the testing of OX5034 stated that:

> "There has been no public consent via a referendum." (0380 p. 2)

Craig Zabransky questioned it is possible to determine whether the "science benefits out weight the risks" when:

> "NO public binding referendum was passed to approve this highly quesitoned [sic] science in the Florida Keys." (0452 p. 1) [Emphasis in Original]

3. The public should be involved in any decisions

Some comments (0378, 0400, 0441) simply argued that the public should be involved in and have a say in any decision making.

Pamm Larry stated:

> "The public should be given the opportunity to be actively involved in the decision-making process and their comments should be taken seriously and have the final say in the decision, as we are the people living here and should have a say in what happens to us and our bioregion." (0441 p. 2)

Florida Keys Environmental Coalition claimed that EPA diverged:

> " . . . from precedent in failing to engage representatives with-standing to meet with the EPA," (0400 p. 2)

Samantha Killhefer stated that:

> "Something like this, should be presented to the public and voted on. You need to find out if the public wants it, not just do it to us. That is NOT okay, and it's unlawful." (0378 p. 1) [Emphasis in Original]

E. Comments Arguing that EPA Needs to Develop New Regulations for GE Insects and Animals

Twelve comments (0383, 0388, 0394, 0396, 0399, 0400, 0401, 0430, 0433, 0434, 0436, 0441, 0461) argued that EPA needs to develop new regulations specific for GE insects and animals. One of these comments (0383) noted that currently no federal agency has formal regulations specific to GE animals and insects and EPA should issue such regulations. Some comments (0383, 0388, 0394, 0401, 0430, 0433, 0434, 0436) argued that only after new regulations are in place should EPA consider applications from potential registrants. Three comments (0396, 0401, 0461) offered suggestions on what those regulations should address and how they should treat privileged information, e.g., information claimed as CBI.

1. <u>EPA should develop new regulations specific for GE insects and animals</u>

Comments in this category (0383, 0388, 0394, 0396, 0400, 0401, 0430, 0433, 0434, 0436, 0441) argued that the EPA should develop formal regulations for GE animals and insects before it allows use of such products. One comment (0383) noted that the U.S. regulatory system is outdated and lacks clear oversight of the use of biotechnology to address insect vectors of animal and human diseases. Some comments (0396, 0399) pointed out that a number of GE insect products are in the development pipeline.

GeneWatch UK stated that:

> "No federal agency has formal regulations specific to GE insects and animals. The current U.S. regulatory system is outdated and lacks clear oversight of the use of biotechnology to address

> insect vectors of animal and human diseases. The EPA should issue new regulations that cover GE mosquitoes before it allows any experimental use of this novel technology." (0383 p. 5)

GeneWatch UK stated that EPA should:

> "Develop new regulations for genetically engineered insects that are designed to be bio-pesticides — only after these regulations are in place should EPA, the State of Florida, and the State of California consider an application for the release of genetically engineered insects;" (0383 p. 7)

The Center for Food Safety stated that:

> "Bio-pesticides as a new class of pesticides should give EPA a chance to pilot a different way of doing business. Risks should not be assessed behind closed doors between company employees and EPA employees. EPA should publish on its website every time its employees meet with advocates of any sort. The Office of Management and Budget already does this." (0396 p. 4)

Similarly, Friends of the Earth stated that:

> "The EPA should develop new regulations for genetically engineered insects designed to be bio-pesticides -- only after these regulations are in place should EPA consider an application for GE insects." (0394 p. 1)

Californians for Pesticide Reform argued that EPA should not "approve this application, nor any future releases of GE mosquitos, until and unless:"

> "The EPA has developed new, robust regulations for genetically engineered insects that are designed to be bio-pesticides;" (0401 p. 4)

Christopher Lish, Judith Borcz, K. Bluefield, Andria Ventura, and Deborah Ramelli stated that EPA should:

> "Develop new regulations for genetically engineered insects that are designed to be bio-pesticides before considering an application for releasing more genetically engineered insects — only after these regulations are in place should the U.S. Environmental Protection Agency, the State of Florida, and the State of California consider an application for the release of genetically engineered insects; and" (0388 p. 2; 0430 p. 2; 0433 p. 2; 0434 p. 3; 0436 p. 2)

2. <u>Comments offering opinions on consequences of EPA not having developed regulations for GE insects and animals</u>

Some comments (0400, 0401, 0441, 0461) offered opinions on the consequences of EPA not having regulations specifically developed for GE insects and animals.

Florida Keys Environmental Coalition stated that:

> "The EPA continues to proceed with examining complex and novel genetically engineered and modified species without any written framework, protocols, or methodology for characterizing and evaluating this technology." (0400 p. 4)

Californians for Pesticide Reform stated that:

> "We share concerns similar to those expressed by The Institute for Responsible Technology that approval of Oxitec's application is a result of a larger failure of the EPA, to date, to adopt proper assessment protocols for approving GMOs." (0401 p. 3)

The Institute for Responsible Technology and Californians for Pesticide Reform stated that:

> "When EPA began to regulate GMOs in the early 1990s, their assessments were already inadequate. Now the technology has far outpaced those insufficient safeguards. We urge you to protect nature, protect the gene pool, and protect our population by rejecting Oxitec's proposed trials and by overhauling your GMO regulations in favor of rigorous independent assessments." (0401 p. 4; 0461 p. 8)

The Florida Keys Environmental Coalition, contending that they "simply want to effect change, so that proper evaluation steps are taken," argued that "Genetic Engineering holds the promise of great possibilities for mankind and at the same time disastrous looms if mistakes are made" yet:

> "The EPA proceeds with this approval as if it was just another pesticide." (0400 p. 6)

The Florida Keys Environmental Coalition further argued that the "ability to assure it is a responsible technology based in honest scientific performance relies on the quality of our regulation. It is a very thin line and a very heavy, but important obligation chosen by a few that are the embodiment of our EPA and our associated regulatory agencies" thus:

> "To proceed with a pattern of arrogant impunity and not provide an investigation of sufficient depth and competence, while a nascent opportunity exists to learn from is wasteful and it is difficult to understand why the EPA would not want to benefit from the knowledge it can gain, to assure subsequent technology evaluations improve. The challenges will only increase and to lower the evaluation bar and not continuously raise the quality of the regulation process seems like the beginning of a classic Greek tragedy," (0400 p. 6)

Pamm Larry stated:

> "I urge EPA to develop more stringent regulations for all types of genetic engineering from the living room hackers to the corporations that patent and profit from them to the universities they fund. Ultimate release of these experimental technologies must be even more regulated." (0441 p. 2)

3. Suggestions on the structure of new regulations

Some comments (0342, 0396) in this category offered suggestions on the risk assessment considerations that should form part of the regulations. Other comments suggested that certain types of information, e.g., information about the human health effects and the effects on the environment and animals in the environment, should not be treated as CBI.

Friends of the Earth stated that:

> "Regulatory action under the Federal 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." (0342 p. 5)

The Center for Food Safety stated that:

> "EPA should start its new regulations by addressing these four areas: 1. Antibiotic resistance promoted by the use of tetracycline to "sterilize" females. 2. Endangered species. 3. Allergenicity. 4. Transparency." (0396 p. 5)

The Center for Food Safety further stated that:

> "Any new regulations must require that all information about the human health effects and the effects on the environment and animals in the environment must be available for public review. In many other countries, health and environmental effects cannot be claimed as "confidential business information"." (0396 p. 9)

The Center for Food Safety contended that EPA should not proceed with these trials until it:

> "Develops new regulations appropriate for the oversight of these new bio-pesticidal insects, including removing claims of confidential business information for environmental and human health effects" (0396 p. 9)

The Center for Food Safety stated that:

> "EPA needs to develop new regulations for genetically engineered insects. Oxitec alone has engineered a dozen or so GE insects. Other companies are proposing to use gene-editing on insect vectors. EPA needs to have clear regulations and not rely on ad-hoc adoption of other pesticide authorities." (0396 p.2)

Meaghan Morrison Hull stated that:

> "Oxitec has myriad other insect "technologies" in their portfolio. Now that they have breached the American market for their "technologies," there will be many more artificially, genetically altered species . . . ," (0399 p. 2)

F. Comments Arguing That EPA Should Ensure Post-Release Control Measures

Five comments (0372, 0397, 0399, 0400, 0461) were received on post-release control measures. Some comments (0372, 0399, 0400) expressed concern that no representatives of the EPA, CDC or the WHO were observed on-site during EUP testing in Florida. Other comments (0397, 0461) pointed out that monitoring should continue post-trial given the length of time that *Ae. aegypti* eggs can remain viable.

1. <u>No representatives of EPA, CDC or WHO observed at the test site</u>

Three comments (0372, 0399, 0400) expressed concern that no representatives of EPA, CDC, or the WHO had been observed at the test site in Florida.

Edward Russo (0372) stated:

> "Where is the oversight in the field to ensure Oxitec is providing accurate information? The people from our local Mosquito Control Board state that people from the CDC, WHO, and the EPA will be down here to provide that "in the field" oversight. That is not true." (0372 p. 1)

Meagan Morrison Hull questioned why:

> "Center for Disease Control does not have any oversight of the Oxitec Mosquito trial in the Florida Keys. CDC will not be conducting any testing, and CDC does not plan to conduct any health assessments before, during, or after the trials." (0399 p. 2)

Florida Keys Environmental Coalition stated that:

> "The EPA has provided, nor are participating in any oversight in the field, bestowing

> complete trust in Oxitec, without concern for the version of "results" the EPA will receive." (0400 p. 2)

2. Post-trial monitoring

Two comments (0397, 0461) were received on post-trial monitoring.

GMO Free Florida stated that:

> "Aedes aegypti eggs may stay viable and resistant to desiccation for up to 450 days. There is no mention of post-trial monitoring of potentially viable eggs for this duration after the trial." (0397 p. 37) [Footnote Omitted]

The Institute for Responsible Technology stated that:

> "EPA has no plans for long term monitoring," (0461, p. 8)

G. Comments Arguing That EPA Must Address Human Testing

Several comments on the applicability of the human studies rules to the EUP testing of OX5034 mosquitoes were received. (0372, 0378, 0380, 0383, 0397, 0399, 0400, 0405, 0412, 0413, 0425, 0426, 0441, 0446, 0451, 0457, 0460, 0461). These comments: (1) argued that informed consent should be part of OX5034 mosquito testing; (2) contested EPA's conclusion that consent is not necessary as Oxitec's research does not meet the regulatory definition of research involving human subjects; and (3) argued that OX5034 mosquito testing should not proceed in light of informed consent considerations.

1. Informed consent should be part of any OX5034 testing

Four comments (0378, 0383, 0397, 0399) argued that informed consent should be part of any testing of OX5034 *Ae. aegypti* mosquito.

GMO Free Florida stated that:

> "Since the release of millions of additional males will likely increase the number of mated females . . . and these mated females require human blood to reproduce and have the offspring of the OX5034, . . . , this must be considered a human experiment. Since humans are directly involved and are required for this experiment, this experiment must abide by all requirements for an experiment on humans including, but not limited to, informed consent." (0397 pp. 12 & 35)

GMO Free Florida stated that:

> ". . . , allergen databases are often incomplete and therefore the risk of an allergic response in residents exposed to the GE mosquitoes is a possibility and residents must be informed of and consent to such a risk." (0397 p. 7)

GMO Free Florida argued that its concerns should be extended to people residing outside of the test areas stating that:

> "With over 3 million visitors to the Florida Keys a year, and numerous vehicles traveling in and out of the area, these genetically engineered mosquitoes escaping the test area is an extremely likely scenario. Since these mosquitoes will likely travel by vehicle to locations outside of the Florida Keys or California, and potentially other countries, the lack of informed consent by communities outside of the Keys and California creates ethical issues. . . . Since the Florida Keys has over 3 million visitors a year the consent of not only the Keys residents, but also the consent of potential tourists must be considered." (0397 p. 35) [Footnotes Omitted]

In support of its position, GMO Free Florida noted that "Darryl Macer, who has published papers on the subject for the World Health Organization and in academic journals" wrote that:

> *"There are a variety of ethical issues that are raised from the use of genetically modified insects . . . . But the most challenging may be the process of informed consent for individuals and communities. Each community or society needs to be given a chance to set consensus.*" (0397 p. 17 & 35) [Footnote Omitted] [Emphasis in Original]

Meagan Morrison Hull stated that:

> "In the 2021 Guidance framework for testing genetically modified mosquitoes, second edition published by the World Health Organization (WHO), recommends risk analysis and public engagement. WHO recommends stringent scientific and ethical considerations to these technologies which will "persist indefinitely." (0399 p. 2)

GeneWatch UK 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." (0383 p .6)

Samantha Killheffer stated that:

> ". . . , what happens if a female lives? And does bite us? . . . . That's NOT informed consent, and that would now be putting human beings at risk, unknowingly which is just UNETHICAL." (0378 p. 1) [Emphasis in Original]

2. Comments contesting EPA's conclusion that consent is not necessary as Oxitec's research does not meet the regulatory definition of research involving human subjects

Three comments (0372, 0383, 0394) contested EPA's conclusion that consent of individuals residing in the testing area is not necessary as Oxitec's research does not meet the regulatory definition of research involving human subjects.

Friends of the Earth argued that EPA's conclusion:

> ". . . is based on Oxitec's claims that female GE mosquitoes won't survive into adulthood. However, there is not publicly available data to support this company claim. There is a risk that female GE mosquitoes will survive and could bite people living in the release areas. . . . It is also possible that people in surrounding areas will be affected. . . . Given these risks, it is critical that all potentially affected communities are given the right to free and prior informed consent to being part of this experiment." (0394 p. 6)

GeneWatch UK, argued that EPA's view is "ethically and legally extremely questionable" because:

> " . . . human subjects will be living in the release areas. They may be bitten by surviving adult female GE mosquitoes, swallow male or female GE mosquitoes, and their risk of mosquito-borne diseases could increase if wild mosquitoes move to a different area, or there is a rebound of *Aedes aegypti* mosquitoes following the releases, or if the competitor species *Aedes albopictus* moves into the area. It is illogical to require consent only if these potential adverse impacts on humans are monitored and assessed." (0383 p. 3)

Edward Russo (0372) stated:

> "This, by definition, is an "experiment," which requires "informed consent" by people used in that experiment. They (you) are taking the position that there will be no impact on humans, endangered species, or our food sources, therefore, no informed consent is needed. That is precisely what part of this experiment is supposed to prove!" (0372 p. 1)

3. Comments arguing that OX5034 mosquito testing should not proceed in light of informed consent considerations

Three commenters (0394, 0397, 0460) argued that the extension and amendment to the EUP should not be granted based on informed consent considerations. Five commenters (0368, 0383, 0400, 0412, 0460)

argued that the risks of the test outweigh the benefits but did not specifically associate the comment with informed consent considerations.

a. Extension and amendment to the EUP should not be granted based on informed consent considerations

GMO Free Florida, referring to the "Declaration of Helsinki" statement that "[M]edical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject (38)", argued that "in this case, . . . , the inherent risks and burdens to the subject outweigh the importance of the objective" because:

> "The objective of this trial is a reduction in mosquitoes, . . . there is no potential reduction in any mosquito-borne diseases . . . . So there is no likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
>
> "Current control methods do not include the release of millions of Aedes aegypti which could potentially increase the risk of mosquito-borne diseases. This makes Oxitec's proposal riskier, with regards to disease spread, than current control methods.
>
> "Current control methods also do not include the potential consumption of synthetic DNA sequences found in Oxitec's mosquito. Gene transfer to intestinal microbiota from food has been observed from the consumption of GE food in humans and such a risk also exists for Oxitec's mosquitoes if swallowed." (0397 p. 33) [Footnote Omitted]

b. Comments arguing that the risks of the test outweigh the benefits but not specifically associating the comment with informed consent considerations

GeneWatch UK contended that:

> "Oxitec's proposed experimental releases will not show whether its GE mosquitoes really . . . . will reduce the numbers of the target wild mosquito (*Aedes aegypti*). Thus, there is no benefit to the proposed experiments and hence the risks discussed below cannot be justified." (0383 p. 2)

Florida Keys Environmental Coalition argued that EPA should review the raw data from the Florida Keys testing and:

> "If there is no benefit demonstrated from the experimentation in the FL Keys, then logic would not support a continuation, or expansion." (0400 p. 3)

Karen Barranco stated that:

> "The uncontrolled experimental release of GMO mosquitoes provides no benefits to any communities in the United States, only risks." (0412 p. 1)

GMO Free Florida added that the Nuremberg Code states that the "voluntary consent of the human subject is absolutely essential" and:

> "A survey of Key Haven residents found 75% oppose GM mosquitoes." (0397 p. 34) [Footnote Omitted]

And:

> "A national survey of 1,211 people found that when people were told about the technology and the risks, more people disagreed with the release of genetically engineered mosquitoes than agreed. An online petition against the release of genetically engineered mosquitoes has over 237,000 signatures including many signatures from people outside of the Keys." (0397 p. 35) [Footnote Omitted]

Friends of the Earth noted that during the "2021 field trials in Florida, residents consistently asked for the trials to stop, for there to be a process of consent and transparency, and for a process of redress" and stated that:

> "The EPA must require Oxitec to obtain the free and informed consent of all potentially affected communities in California and Florida before any trial is allowed to move forward, and mechanisms should be made available to halt the experiment if the community demands." (0394 pp. 5 & 7)

Friends of the Earth stated that:

> "There should be communication in multiple languages throughout the process of assessment 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." (0394 p. 6) [Footnote Omitted]

Comment 0460 representing Mass Mailer stated that:

> "People in the trial areas have not provided their consent to being experimented on with GMO mosquitoes." (0460 p. 2)

H. Comments Addressing Other Ethical Issues

Other concerns expressed in comment revolving around ethical considerations focused on: (1) how OX5034 could affect communities with environmental justice concerns, and (2) how use of OX5034 might affect the natural world.

1. Environmental Justice Considerations

Two comments (0415, 0421) offered an opinion on how GE mosquitoes could be beneficial to communities below the poverty line. Two comments (0396, 0451) referring to the EUP testing of OX5034 expressed concerns in the context of environmental justice - one comment laid particular emphasis on Latin-X communities while the other made particular reference to Black, Indigenous, People of Color (BIPOC).

a. GE mosquitoes could be beneficial to communities below the poverty line

Two comments (0415, 0421) offered an opinion on how GE mosquitoes could be beneficial to communities below the poverty line.

Leor Kaminski stated that:

> "Aedes aegypti likes to lay its eggs in untreated standing water, which is more common in areas of poverty. While more research needs to be conducted on the link between poverty and Aedes aegypti, the approval of a permit extension may lead to some environmental justice by aiding in the control of Aedes aegypti as a virus vector which may disproportionally affect communities with high levels of poverty." (0415 p. 1) [Footnote Omitted]

Lawrence Hribar stated that:

> "The company is investigating an environmentally friendly method of pest control that has no effect on nontarget organisms and reduces pesticide use, especially in minority communities who often bear the brunt of mosquito-borne disease outbreaks." (0421 p. 1)

b. Comments expressing concern about OX5034 testing in the context of environmental justice

Two comments (0396, 0451) referring to the EUP testing of OX5034 expressed concerns in the context of environmental justice.

The Center for Food Safety stated that:

> "The public needs to know how the risks and benefits of these decisions will likely impact us all, and certain communities, especially those of BIPOC communities even more so. The COVID-19 pandemic has made it clear that robust public health depends on informed communities who feel that they are being invited to participate in collective actions. Release of GM mosquitoes is no different. For the health of ourselves, the nation, our planet, and future generations, environmental regulation of GMOs must be made more rigorous and just." (0396 p. 5)

Bruce Campbell stated that:

> "I OBJECT ON ENVIRONMENTAL JUSTICE GROUNDS (among others) TO THE PROPOSED GMO MOSQUITO RELEASE. I NOTE THAT SOME OF THE CENTRAL VALLEY AND INLAND COUNTIES have LARGE LATIN-X POPULATIONS who are EXPOSED TO SERIOUS LEVELS OF TOXIC MATERIALS ALREADY, and do not need TETRACYCLINE-RAISED MOSQUITOES on top of their other problems to prompt some to DEEM THE CENTRAL VALLEY an "APPALACHIA" in terms of POVERTY & TOXIC EXPOSURES. . . . Do not add to their woes with this secret scheme to further throw the Central Valley ecosystem (which used to be a Serenghetti but now is a toxic stew of chemical ag, fracking, dumpsites, and diesel) out of balance on microscopic levels. . . ." (0451 p. 1) [Emphasis in Original]

2. Preservation of the Natural World

Some comments (0373, 0378, 0379, 0380, 0400, 0406, 0409, 0420, 0438, 0410, 0441, 0444, 0448, 0450, 0453, 0456) raised issues revolving around preservation of the natural world. These included: (1) ethical concerns about implementing any strategy that might result in changes to the gene pool, including elimination of a species; (2) appropriateness of attempting to reduce any insect population in light of the decline in insect populations; and (3) comments on the need for the Agency to proceed cautiously in its decision-making.

a. Ethical concerns about implementing any strategy that might result in changes to the gene pool, including the elimination of a species

Some comments (0373, 0378, 0379, 0380, 0406, 0409, 0420, 0438, 0456) raised ethical concerns about interfering with the population of a species, including a comment on extinction. EPA also received three comments (0373, 0409, 0456) expressing the opinion that a genetically engineered organism is unnatural.

Samantha Killheffer stated that:

> "This is basically - purposefully extincting a species from the planet. That doesn't sound like "protection" of the environment at ALL. That just sounds horrible, and like something nobody should ever be allowed to do. If no mature females survive, this species will never reproduce

> ever again, and We/Oxitec will have wiped them out. I thought we were trying to "protect" the environment and the animals, including insects, living in that environment, with your agency. . . . It is a very slippery slope when you start giving and taking life wherever, or whenever one feels the urge, as if nature wasn't SACRED. We are not Gods, and we should not be trying to "play God" with any animals, insects, or ANY living things. Nature is to be protected, not killed off." (0378 p. 1) [Emphasis in Original]

Tim Gordon stated that:

> "GE tampering with nature is going to have untold horrible results way down the road." (0379 p. 1)

Anonymous (0406) stated that:

> "Why do men seem to feel the need to control everything?! What disconnect do men have to feel the need to manipulate and rape Mother Nature the way they have and continue to do . . . ." (0406 p. 1)

Shelly Ready stated that:

> "You are tinkering with Mother Nature and destroying our futures in ways we cannot even comprehend yet. This will affect generations to come and we are responsible for making our world better, not worse." (0438 p. 1)

Frances Micklam stated:

> " . . . protecting the gene pool is a global issue and the spread of organisms knows no boundaries, as we learnt from the pandemic. The only time to stop the spread of GE organisms is before they are released." (0380 p. 2)

Jo Rand stated that:

> "Why must you mutate the planet with franken species? We already eat frankenfood... isn't that enough?" (0373 p. 1)

Jessica Denning stated that:

> "These frankenbugs are the ultimate microinnocculators. Mosquitoes inject spit into their hosts, as part of their routine." (0409 & 0456 p. 1)

b. Appropriateness of attempting to reduce any insect population in light of the decline in insect populations

One comment (0441) expressed concern about reducing any insect population in light of the general decline of insect populations.

Pamm Larry stated that:

> "We have already seen a terrifying reduction in our bug population in Butte County, where I live, and the other counties I have been through in California within the past few years. Until around four years ago, my windshield would be thick with flying insect carcasses after driving through the rice fields to the point where vision was difficult. Now, there are literally around 20. This is an alarming trend that threatens entire wildlife food chains." (0441 p. 1)

c. Comments urging the Agency to proceed cautiously in its decision-making

Seven comments (0400, 0410, 0441, 0444, 0448, 0450, 0453) urged EPA to exercise caution in its decision-making process.

Julie Ostoich stated that:

> "The EPA and all our agencies need to use the Precautionary Principle. There is no long term evidence or testing that shows this is safe." (0410 p. 1)

Pamm Larry stated that:

> "I wish that the Precautionary Principle would be applied as opposed to the continued regulatory support of profit and furtherance of exciting, but potentially dangerous, technology. It is apparent after decades of mistakes that have taken a huge toll on our world, that we need to consider the impact of what we do more than we have." (0441 p. 1)

Susan Brierley stated that:

> "California is suffering enough with drought and wildfires! Don't make this go any further than Florida! Please, please, please! I'm only 71 years old, but have lived through many man made environmental disasters and we can't take any more chances!" (0444 p. 1)

Belinda Mostert stated that:

> "We work hard to balance a fragile eco-system here…please respect our home!" (0453 p. 1)

Ariane Glazer urged that EPA:

> "!!!!!! Use the precautionary principle. . . ." (0450 p. 1)

Florida Keys Environmental Coalition stated that:

> "Harm . . . could remain unnoticed for years. . . . Learning to appreciation the true sensitivity of any ecosystem or the delicate symbiosis required for balance is a never-ending pursuit. . . . " (0400 p. 5)

I. Comments Arguing EPA Must Prepare a Full EIS Under the National Environmental Policy Act

Some comments (0383, 0394, 0396, 0397, 0405, 0414, 0451, 0460) argued that EPA must prepare a full Environmental Impact Statement (EIS) under the National Environmental Policy Act (NEPA). Some comments (0383, 0394, 0397) argued that EPA should prepare an EIS because: (1) OX5034 mosquito may present complex environmental issues that may not be adequately addressed under FIFRA, and/or (2) an EIS would include consideration of the EPA's responsibilities under other environmental legislation. Other comments (0383, 0394) described specific questions that the commenters believed would be better addressed in an analysis under NEPA. Some comments (0394, 0396, 0397, 0405, 0451, 0460) simply indicated that the EUP amendment and extension should not be granted without EPA preparing an EIS evaluation.

1. Comments arguing that EPA should prepare an EIS

Comments in this category (0383, 0394, 0397) argued that EPA should prepare an EIS because: (1) OX5034 mosquito may present complex environmental issues that may not be adequately addressed under FIFRA, and/or (2) an EIS would include consideration of the EPA's responsibilities under other environmental legislation.

Friends of the Earth 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 be required." (0394 p. 5)

Friends of the Earth stated 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 risks, and analyze alternatives to these actions. As part of these requirements, the EPA should

> undertake a full EIS so that it may thoroughly examine the potentially substantial impacts that the proposed action may have." (0394 p. 5)

GMO Free Florida 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." (0397 p. 38)

The Center for Food Safety stated that "[G]iven the proposed extension of time for trials in Florida and the proposed expansion to include releases in California:

> ". . . there is even more need here for EPA to take a closer look and to require additional information and consult with other federal agencies prior to authorizing these expanded releases of experimental living organisms into the environment. As explained in previous comments, EPA must comply with other laws including the National Environmental Policy Act and the Endangered Species Act and thus should prepare an Environmental Impact Statement and consult with the U.S. Fish and Wildlife Service and National Marine Fisheries Service before deciding whether to approve the proposal at issue here. The necessity to do so is even more important with the expanded release proposed here, that includes a vast increase in the acreage for releases and expansion to releases in California, which is home to numerous federally threatened and endangered species, as well as areas of dense human populations." (0394 p. 2)

2. <u>Specific questions commenters believe would be better addressed under NEPA</u>

Comments in this category (0383, 0394) revolve around requests that an EIS be performed for each county Oxitec has identified as a potential testing site for OX5034, including in relation to possible widespread application and presence of tetracycline in the environment, and that the EIS identify further studies that should be performed.

Friends of the Earth, urging "the EPA to conduct a full EIS" stated that:

> "Oxitec's application does not consider the complexity of ecosystems carefully enough, nor the vast diversity of California's ecosystems across the state. A complete EIS in each county 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." (0394 p. 3)

Friends of the Earth stated that:

> "There should be a full Environmental Impact Statement (EIS) under the National Environmental Policy Act (NEPA) for each of the proposed counties, and this should be reviewed by a committee of independent ecologists and entomologists, public health experts, and other key experts and public stakeholders." (0394 p. 1)

Friends of the Earth further stated that the "possible widespread application and presence of tetracycline in the environment could significantly undermine the efficacy of GE mosquitoes," thus:

> "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." (0394 p. 3)

GeneWatch UK, arguing that "considerable further evidence is needed to assess whether the use of the pesticide under the proposed permit (including its method of delivery via GE mosquitoes) may cause unreasonable adverse effects on the environment," stated that:

> "As well as considering the legal obligations . . . , the EPA should therefore specify that further studies be conducted before publishing a full EIS for public consultation." (0383 p. 7)

3. <u>Comments against granting the EUP amendment and extension without preparing an EIS</u>

Comments in this category (0394, 0396, 0397, 0405, 0451, 0460) argued that EPA should not grant the amendment and extension unless the Agency prepares an EIS.

Friends of the Earth, "in light of the unanswered questions and the gaps in data analysis" urged EPA to reject Oxitec's amendments and extension requests, and stated that:

> "EPA should request further studies from Oxitec and require a full EIS be published for public consultation ahead of an application for an EUP." (0394 p.7)

GMO Free Florida stated that "EPA must reject Oxitec's application for genetically engineered mosquitoes and":

> "Complete a full environmental impact statement on Oxitec's GMO mosquito release proposal;" (0397 p. 37)

The Center for Food Safety contended that EPA should not proceed with these trials until it:

> "Prepares an Environmental Impact Statement." (0396 p. 9)

Comment 0460 representing Mass Mailer, Bruce Campbell and Penelope McMillan opposed Oxitec's request for an amendment and extension of its EUP for OX5034, because:

> "There has been no Environmental Impact Statement." (0405 p. 1; 0451 p. 2; 0460 p. 2)

J. Comments Regarding the Endangered Species Act

Some comments (0383, 0396, 0405, 0460, 0446) stated that EPA must comply with the Endangered Species Act (ESA), with one comment (0396) offering its characterization of the general requirements of the ESA. Other comments (0383, 0405, 0446, 0451, 0460) offered general statements on the need for compliance with the ESA. Overall, the comments regarding the ESA argued that EPA did not complete an endangered species analysis; that EPA's endangered species analysis was inadequate; or that EPA did not properly consult with the U.S. Fish and Wildlife Service (FWS) or the National Marine Fisheries Service (NMFS) (collectively, "the Services").

1. Comments Stating That the EPA Must Comply with the ESA

Comments in this category (0383, 0405, 0446, 0451, 0460) simply voiced the opinion that EPA should comply with ESA requirements. One comment in this category (0396) offered a characterization of the general requirements that the ESA places on Federal agencies.

The Center for Food Safety described the requirements the ESA places on federal agencies:

> "Section 7 of the Endangered Species Act (ESA) requires federal agencies such as EPA, in consultation with the expert wildlife agencies, to ensure that any action authorized, funded, or carried out by the agency is not likely to jeopardize the continued existence of any threatened or endangered species, or result in the destruction or adverse modification of the critical habitat of such species. If the action agency determines the action "may affect" a listed species or critical habitat, the action agency must formally consult with NMFS and/or FWS to "insure" that the action is "not likely to jeopardize the continued existence" of that species, or "result in the destruction or adverse modification of habitat … determined … to be critical…." The threshold for a finding of "may affect" is extremely low. A triggering effect need not be significant; rather "any possible effect, whether beneficial, benign, adverse, or of an undetermined character, triggers the formal consultation requirement…." During consultation, EPA is prohibited from making any irreversible or irretrievable commitment of resources with respect to the agency action which may foreclose the formulation or implementation of any reasonable and prudent alternative measures." (0396 p. 6)

GeneWatch UK stated that:

> ". . . , the EPA should ensure that it is complying with the Endangered Species Act." (0383, p. 5)

Comment 0460 representing Mass Mailer, Bruce Campbell and Penelope McMillan stated that:

> "There has been . . . no endangered species assessment. There are approximately 200 endangered species in California." (0405, p. 1; 0451 p. 2; 0460 p. 2)

Keith Schildt stated that "[O]nce GE mosquitos are released into the wild, there is no calling them back" and:

> "I could not find if an endangered species assessments have ever been done on the release in Florida." (0446 p. 1)

2. Comments Stating That EPA Should Consult with FWS or Other Experts

The Center for Food Safety disagreed with EPA's 2020 "no effect" finding for the OX5034 mosquitoes EUP and stated that consultation with wildlife agencies such as U.S. Fish and Wildlife Service must occur:

> "In approving the release of Oxitec's OX5034 GE mosquitoes with the original EUP, EPA made erroneous and unilateral assumptions that its action would have "no effect" on protected species and/or their critical habitat.[vi] Like its approval decision, EPA's conclusion concerning threatened and endangered species rests on an extremely limited inquiry that failed to adequately consider the significant risks of harm to listed species related to releasing more than a billion GE mosquitoes into the environment at the test trial sites in Monroe County and Harris County. […] EPA failed to conduct a proper ESA analysis for the original EUP application and has done nothing to correct that error, not to attempt to comply with the ESA with this proposed amendment and extension. EPA must work with the expert wildlife agencies, U.S. Fish and Wildlife Service and National Marine Fisheries Service, to properly examine potential impacts to protected species and to consult as necessary under the ESA." (0396 p. 6)

The Center for Food Safety stated that:

> "The risk assessment shows no evidence that EPA engaged with the Fish and Wildlife Service to consider the endangered species impacts in Florida and Texas. Instead, EPA made an unsubstantiated claim and misapplied the Endangered Species Act in concluding that these GE mosquitos would have no effect on endangered and threatened species." (0396 p. 3)

The Center for Food Safety stated that:

> "Clear consultation with both other federal agencies and with local agencies that know about endangered species should be a part of regulatory requirements for GE insects." (0396 p. 7)

The Center for Food Safety contended that EPA should not proceed with permitting the EUP amendment and extension until it:

> "Consults with other federal and state agencies with more expertise in endangered species in the locations where GE insects will be released, and specifically conduct a proper ESA consultation with the U.S. Fish and Wildlife Service and National Marine Fisheries Service for the releases currently proposed in Florida and California." (0396 p. 9)

3. Comments stating a lack of ESA analysis for California

Some comments (0396, 0401, 0413) stated that no ESA analysis had been completed for the proposed counties in California where testing of OX5034 may occur.

John Ulloth (0413) stated that:

> "There has been . . . no endangered species assessment. There are approximately 200 endangered species in California." (0413 p. 2)

Californians for Pesticide Reform stated that:

> "California is a global biodiversity hotspot and is home to more species of plants and animals than any other state in the nation yet there is no discussion on the potential impacts on the unique ecosystems and endangered species habitat in California. Nor does the docket discuss the significant agricultural lands in California and the associated chemicals used in these settings, such as tetracycline, that would impact any field trial." (0401 p. 1)

Californians for Pesticide Reform added that:

> "Importantly, no information regarding populations of *Aedes aegypti* in California has been provided, nor any evidence regarding competitor species or predators/prey. This makes it impossible to assess environmental risks." (0401 p. 2)

The Center for Food Safety stated that:

> "Now, in considering the proposed amendment and extension of that EUP, EPA has done no additional analysis under the ESA, despite the expansion of releases over a large swath of the state of California, which is home to numerous federally protected threatened and endangered species." (0396, p.6)

4. Comments discussing what locations and which species should be included in the ESA assessment

Two comments (0396, 0397) stated the locations in which they believed testing of OX5034 would occur, and therefore, based on the locales, which species should be included in the ESA assessment.

GMO Free Florida challenged Oxitec's claim for OX513A that *"The Stock Island Tree Snail is the only species found in the physical vicinity of the proposed trial site"* arguing that:

> ". . . they ignored that mosquitoes are capable of getting in a car, boat, or other means of transport, these mosquitoes are capable of leaving the physical vicinity of the proposed trial site and could even travel as far as another country. Therefore, Oxitec must consider this and, at a minimum, assess risk for all threatened, endangered, or candidate species that were identified in Monroe County. (0397 p. 22) [Footnotes Omitted]

GMO Free Florida specifically argued that Key deer should be considered in an assessment, as the *"National Key Deer Refuge headquarters is located on Big Pine Key, which is 100-miles south of Miami and 30 miles north of Key West on Highway US-1, and 26 miles from Key Haven"* because:

> "Since Oxitec's mosquitoes can enter a vehicle and that vehicle can easily travel 26 miles, this type of scenario is very likely." (0397 p. 22)

GMO Free Florida argued that because OX5034 can be transported from the release site, a risk assessment must be performed:

> " . . . for all threatened, endangered, or candidate species that were identified in Monroe County. For example, endangered or threatened reptiles in Florida Keys that consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include *Eumeces egregius egregius* and *Eumeces egregius insularis*. Endangered or threatened birds in Florida Keys that consume mosquitoes, eggs, larvae, pupae or adults, or consume other species which consume mosquitoes include *Ammodramus maritimus mirabilis*, *Vermivora bachmanii*, *Calidris canutus rufa* and *Charadrius melodus*." (0397 p. 22)

GMO Free Florida stated that for releases in California:

> "Oxitec must also assess the risk of all endangered species in the state of California. Besides *Ambystoma californiense* a number of endangered amphibians in California are also very likely to consume large amounts of *Aedes aegypti*, eggs, larvae, pupae or adults, or consume other species which consume mosquitoes, these include, but are not limited to: *Ascaphus truei, Batrachoseps sp, Batrachoseps campi, Batrachoseps pacificus pacificus, Batrachoseps relictus, Batrachoseps simatus, Batrachoseps stebbinsi, Bufo canorus, Bufo exsul, Bufo microscaphus californicus, Ensatina eschscholtzii croceator, Ensatina eschscholtzii klauberi, Hydromantes sp, Hydromantes brunus, Hydromantes platycephalus, Hydromantes shastae, Plethodon elongatus, Plethodon stormi, Rana aurora aurora, Rana aurora draytoni, Rana boylii, Rana cascadae, Rana muscosa, Rana pretiosa, Rana yavapaiensis, Rhyacotriton variegatus, Scaphiopus hammondii*. Considering, for example, that several *Bufo* are endangered in California and *Aedes aegypti*

> prefer to lay their eggs in water sources with tadpoles of the genera *Bufo* in them this means that the subjects used for toxicity testing by Oxitec are inadequate to assess the safety of amphibians that will almost definitely consume large amounts of OX5034 if released." (0397 p. 23) [Footnote Omitted]

GMO Free Florida stated that similarly for reptiles:

> "Endangered or threatened reptiles in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Anniella pulchra nigra, Anniella pulchra pulchra, Clemmys marmorata marmorata, marmorata, Cnemidophorus hyperythrus, Cnemidophorus tigris multiscutatus, Coleonyx switaki, Coleonyx variegatus abbotti, Elgaria panamintina, Eumeces skiltonianus interparietalis, Heloderma suspectum cinctum, Sceloporus graciosus graciosus, Sceloporus graciosus vandenburgianus, Uma notata notata, Xantusia henshawi gracilis, Xantusia vigilis sierrae*. Toxicity testing with reptiles consuming OX5034 is absolutely necessary considering the risk to endangered species." (0397 p. 23)

GMO Free Florida stated similarly for insects:

> "Endangered or threatened insects in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Capnia lacustra, Ammopelmatus kelsoensis, Ammopelmatus muwu, Macrobaenetes kelsoensis, Ambrysus funebris, Pelocoris shoshone, Agabus rumppi, Chaetarthria leechi, Hydroporus hirsutus, Hydroporus leechi, Hydroporus simplex, Hygrotus curvipes, Hygrotus fontinalis."* (0397 p. 22-23)

GMO Free Florida stated similarly for birds:

> "Endangered or threatened birds in California that may consume mosquitoes, eggs, larvae, pupae or adults, or eat other species which consume mosquitoes include, but are not limited to: *Agelaius tricolor, Aimophila ruficeps canescens, Amphispiza belli belli, Chlidonias niger, Geothlypis trichas sinuosa, Histrionicus histrionicus, Ixobrychus exilis hesperis, Laterallus jamaicensis, Plegadis chihi, Toxostoma lecontei macmillanorum."* (0397 p. 22-23)

The Center for Food Safety expressed concern:

> "California has a tremendous number of endangered species that should be considered before billions of OX5034 mosquitoes are released in California. Without information about the specific counties in California where the mosquitos are proposed to be released, it is difficult to pinpoint which endangered species are of particular concern. However, there are likely to be many

> species that should be considered, such as listed species like the California red-legged frog, the California tiger salamander, and the vernal pool fairy shrimp" (0396 p. 3)

The Center for Food Safety added that it had previously expressed concerns:

> ". . . about potential impacts to threatened and endangered species in Monroe County, Florida. These concerns remain, and the same concerns apply to those protected species in California. Given the large amount of acreage proposed for releases in California and a potentially large number of counties where releases may take place (up to 20 based on Oxitec's application) there are many more species that may be put at risk through approval of these additional releases. California is a state with a wide variety of habitat types, high levels of biodiversity, and home to many threatened and endangered species." (0396 p. 6-7)

K. Comments Questioning Whether Oxitec Can Be Released from the Contained Use Requirements of an Import Permit

Four comments (0380, 0383, 0394, 0397) requested clarification of the legal basis allowing open release of OX5034 mosquito under an EUP issued under FIFRA, given CDC import regulations for infectious biological agents, infectious substances, and vectors (42 CFR 71.54).

GeneWatch UK 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." (0383 p. 5)

GeneWatch UK, noting that "Evans et al. (2019) highlights the spread of genes from Oxitec's previous strain of GE mosquito, OX513A, into the wild population from previous experiments in Brazil" and that EPA stated that this "is not related to the CDC import permit" (page 140), argued that EPA's position:

> ". . . , is questionable because the import permit is what prevents the release of this imported nonnative mosquito strain into the environment. Such strains (whether or not they are genetically engineered) are usually required to be studied only in contained use, due to the risks highlighted in the Evans et al. paper, particularly the risk of increasing the capacity of wild *Aedes aegypti* mosquitoes to spread disease, by introducing genetic changes not present in the local mosquito population. This risk is likely to be much higher with the new OX5034 strain than with the OX513A strain studies in Brazil, . . . , because the new strain is female-killing only." (0383 p. 4)

GMO Free Florida stated that:

> "As a first step, the EPA should 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 insects to be deliberately released into the environment." (0397 p. 38)

Friends of the Earth 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." (0394 p. 5)

Frances Micklem expressed concern about:

> "Failure to follow transboundary notification processes for exports of GM insects correctly;" (0380 p. 1)

L. Comments on Economic Considerations

Several comments (0388, 0396, 0397, 0399, 0434, 0436, 0439, 0452, 0461) addressed economic considerations they believe to be associated with release of the OX5034 mosquito. Some comments speculated on potential effects of introduction of OX5034 mosquito on taxpayers and tourism. One commenter brought up the question of liability.

1. Costs to Taxpayers

Three comments (0396, 0399, 0439) speculated on potential direct costs to the taxpayer of OX5034 mosquito.

The Center for Food Safety stated that:

> "EPA approval of the release of this new GE mosquito is just the first step toward Oxitec selling its proprietary mosquito to US mosquito-control boards, and by extension taxpayers who fund mosquito control." (0396 p. 3)

Meaghan Morrison Hull stated that:

> "We were not even provided a cost comparison study, and our tax dollars are paying for the FKMC components of the experiment. FKMC, a government agency, is providing staff, labor, trucks, and technology for this "FREE" experiment." (0399 p. 2) [Emphasis in Original]

Anonymous (0439) stated that:

> ". . . . , GE mosquito production is costly. In 2014, the release of 300,000 GE mosquitoes in Panama was reported to have cost $620,000 (more than $2 per mosquito)." (0439 p. 1) [Footnote Omitted]

2. Concerns About Potential for Effects on Tourism

Several comments (0388, 0434, 0436, 0439, 0452, 0461) expressed concern about the potential for releases of OX5034 mosquito to adversely affect tourism. Some of these comments argued for more testing and/more public outreach based on their concerns about potential of OX5034 to affect tourism.

Christopher Lish, Judith Borcz, Andria Ventura and Deborah Ramelli stated OX5034 releases:

> " . . . may also impact California's economy, which is one of the largest in the country, and Monroe County's economy, which depends on tourism." (0388 p. 2; 0434 p. 2; 0436 p. 2; 0439 p. 1)

The Institute for Responsible Technology noted economic dislocations that arose with genetically engineered food in international commerce and tied that experience to potential effects on tourism:

> "Many jurisdictions in the world have zero tolerance for unapproved genetically engineered contamination in food. When unapproved varieties of genetically engineered corn, rice, and flax were found, shipments were rejected, markets were closed, and billions of dollars were lost. In some cases, export markets closed their doors to all shipments of corn, rice or flax from the entire country where the contamination occurred. If a non-GMO supplier wanted to reestablish trade, the burden of proof was typically on the supplier to sample, test, and sometimes gain third party certification. This can be expensive and time-consuming. To confirm GMO contamination, sample materials are often blended and then tested using PCR. If the lab uses methods designed to detect GE mosquitoes, even a tiny amount of contamination can be identified. EPA can claim that the probability of an actual or perceived health issue, or economic loss due to contamination, is quite low. But EPA hasn't actually consulted with the businesses in the regions impacted by these events. Most tourist businesses in South Florida remain unaware of the risks to their industry by the GE mosquitoes already released because EPA has refused to engage them in the risk assessment." (0461 p. 6)

Craig Zabransky expressed concern about the potential effects of OX5034 testing in the Florida Keys on tourism, particularly as the Keys works to overcome recent economic challenges, e.g., the effect of the global SARS-CoV-2 pandemic on tourism:

> "This largely untested technology and relatively unknown is a BIG risk to our continued recovery. . . . April was a record month for tourism but now we face a new self-imposed threat, the recent release of genetically modified mosquitoes. . . . Let's not risk the future of the Florida Keys on an unneeded, unproven, and untested new technology at this critical time. Let's wait until we know more about them and their safety in our environment." (0452 p. 1 attachment) [Emphasis in Original]

The Institute for Responsible Technology, contending that release of GE mosquitoes could have adverse economic impacts on tourism should there be actual or misperceived health problems, stated that:

> "Even if the GE mosquito did not cause harm, the misperception that it does can impact the tourist economy. . . . . This threat is exacerbated by the lack of safety testing." (0461 p. 5)

The Institute for Responsible Technology further contended that:

> "If bites from GE mosquitoes released in Florida or California were found to cause serious health issues, the impact on tourism could be catastrophic, especially if the GE mosquitoes persisted in the environment." (0461 p. 5)

3. Liability/Insurance

While arguing that more testing is needed, one comment (0397) also argued Oxitec should inform the public of how the company intends to deal with the issues of liability and insurance.

GMO Free Florida, arguing that female OX5034 mosquitoes could still be present in the environment post-testing and bite someone, and moreover could move from the test site to other locations, stated that:

> "Oxitec must therefore put aside funds to deal with the possibility of lawsuits in the case of such a scenario. It should also be mandatory that Oxitec is insured in the case that these, or other, events occur. These details are also missing from the documentation. Since Oxitec admits, '*Uncertainty can be reduced by obtaining or generating more data on particular aspects*' they should have no problem conducting the suggested studies to generate more data and reduce uncertainty before releasing these GE mosquitoes into the environment in the Florida Keys." (0397 p. 37) [Footnote Omitted] [Emphasis in Original]

M. Comments on Disease Incidence and Recommendations that Oxitec Apply to FDA

Some comments (0394, 0396, 0397) claiming that Oxitec in some of its advertising and other materials claimed that OX5034 mosquito could be used to control disease, argued that testing under the EUP should include epidemiological studies on disease transmission. Some of these comments argued that

Oxitec should apply to the FDA for approval for products having disease reduction claims. Some comments (0368, 0413, 0418, 0451, 0454, 0460), however, argue that the diseases vectored by *Ae. aegypti* are not a problem in the US.

1. Oxitec has Made Disease Claims for OX5024 and Disease Reduction Should be Tested

Some comments (0394, 0396, 0397, 0451) argued that Oxitec has made disease claims and, as the EUP testing does not include epidemiological studies, should apply to FDA.

The Center for Food Safety stated that:

> "Oxitec claims its strategy is a "safe" and "environmentally sustainable" and "friendly" way to control mosquitoes that transmit disease. Yet the trial that the EPA is conducting is not one that assesses the control of disease. Oxitec would have to apply to the FDA for that review." (0396 p. 3)

GMO Free Florida stated that on occasion:

> "Oxitec has clearly made a disease claim as is defined by the FDA. However, it appears they have not followed the law in doing so. Therefore, the EPA must urge the FDA to investigate Oxitec for violation of the law and for the FDA to require approval of these genetically modified mosquitoes as Oxitec is clearly making claims of disease reduction which is outside of the EPA's jurisdiction." (0397 p. 36)

Friends of the Earth stated that:

> "Oxitec's rationale is based on an assumption that mosquito population reduction will reduce or eradicate diseases such dengue and zika. However, Oxitec has not provided the EPA data to support this claim. 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. Oxitec should provide a specific mechanism through which its proposed releases might reduce the risk of diseases spread through mosquitoes. Without this information, Oxitec's proposed 'pesticide' experiment will not address disease reduction." (0394 p. 4) [Footnote Omitted]

Comment 0460 representing Mass Mailer and Bruce Campbell stated that:

> "Oxitec refuses to provide data and has not proven that their GMO mosquitoes reduce disease transmission. Oxitec has even admitted that the Florida Keys experiments will only track mosquito populations before and after GMO mosquito releases, not disease suppression or transmissions." (0451 p. 2; 0460 p. 2)

2. Low Incidence of *Ae. aegypti* Vectored Diseases in the US Makes OX5034 Unnecessary

Some comments (0368, 0378, 0380, 0418, 0451, 0460) argued that OX5034 is not needed as the incidence of diseases vectored by *Ae. aegypti* is low in the US.

Bruce Campbell stated that:

> "GMO mosquitoes are a risky technology created to solve a problem that is negligible in the United States. In California, there have been ZERO cases of locally acquired dengue. As a matter of fact, there have only been 332 dengue cases in the entire U.S. in 2020, of which only 80 were locally transmitted cases, according to the CDC. The rest were travel-associated, contracted outside of the U.S. The CDC says, 'Yellow fever is a very rare cause of illness in U.S. travelers.' Yellow fever can be transmitted by Aedes aegypti to travelers in Africa and South America. And what about malaria? Malaria isn't even transmitted by Aedes aegypti, it's transmitted by Anopheles mosquitoes. So malaria is irrelevant." (0451 p. 2) [Emphasis in Original]

Making the same argument, comment (0460) representing Mass Mailer added that:

> "Dengue fever has affected 110 people in Florida in 2020. But only 70 were locally-transmitted cases. The last small outbreak in Florida occurred in 2013." (0460 p. 1)

Dylan Russell stated that:

> "I don't see any significant cases of yellow fever reported in the US since 1905, there were 5K travel reported cases of dengue fever in the US in 2020, no confirmed zika cases in the US in 2020, 29 reported cases of chikungunya virus in the US in 2020. These are NOT viruses we suffer from in the US." (0368 p. 1) [Emphasis in Original]

Samantha Killheffer stated that:

> "We have not had ANY cases of Zika, Dengue fever, Ebola, West Nile, Yellow Fever etc... of late. Perhaps we've had less than 10 cases of Yellow Fever in the last 10 years, if that.
> So using the excuse/reason that "we need to wipe these mosquitoes out because they carry those illnesses and could possibly give them to humans" doesn't really hold any water. The possibility of infection is SO minute, that it does not give even close to a good enough reason to release all these GMO mosquitoes here, nor reason to wipe out a whole species. We are not having problems with those illnesses around here, and some of them we NEVER see." (0378 p. 1) [Emphasis in Original]

N. EPA Should Not Rely on the Registrant Provided Assessment Data/Information

Several comments (0388, 0396, 0397, 0400, 0401, 0418, 0430, 0433, 0434, 0436, 0441, 0461) argued that EPA should not rely on registrant provided data/information.

The Center for Food Safety stated that:

> "To ensure rigorous review, the EPA and other regulatory bodies must also fund independent third-party research on GM mosquitoes and their potential impact on US ecosystems and human health. Potential risks are too important to be left to corporations alone to research, and the American public needs to be assured that these decisions are made free of conflicts of interest." (0396 p. 4)

Californians for Pesticide Reform and Judith Borcz stated that:

> "Our elected officials and government agencies must not rely solely on data from the companies that would profit from genetically engineered organisms to decide what information the public and regulators should know or what is considered safe." (0401 p. 4; 0430 p. 2)

Californians for Pesticide Reform, Christopher Lish, Judith Borcz, Andria Ventura, K. Bluefield and Deborah Ramelli stated that:

> "Sound science cannot be based on corporate confidential business protections but must be reviewed objectively by independent scientists and made available to the public ahead of any releases into the environment. This is basic to the scientific method and common sense and necessary to ensure the health of the public and the environment are protected." (0388 p. 2; 0401 p. 2; 0430 p. 2; 0433 p. 1; 0434 p. 2; 0436 p. 2)

Jerry Russell stated that:

> "EPA and/or NGO's should sponsor independent research to provide verification of corporate-sponsored studies." (0418 p. 1)

Pamm Larry stated that:

> "We need environmental impact reports, transparency on the existing data collection all interpreted by independent scientists, not Oxitec itself." (0441 p. 1)

The Institute for Responsible Technology stated that:

> "EPA relies on companies to conduct or commission their own studies on the products being assessed. Investigations confirm that companies often design their research and cherry-pick results to force a conclusion of safety." (0461 p. 3)

GMO Free Florida, Christopher Lish, Andria Ventura and Deborah Ramelli stated that:

> "Our government agencies must not rely only on data from companies that would profit from genetically engineered organisms to decide what information the public and regulators should know." (0388 p. 2; 0397 p. 38; 0434 p. 3; 0436 p.2)

Florida Keys Environmental Coalition stated that:

> "For profit companies have simple short-term goals, make more money and increase shareholder value. There are cases where those short terms goals lead to diabolical outcomes because of the bad decisions business and the people in them make. The creation of EPA was a means to help prevent those damages to humans and ecosystems when it was formed." (0400 p. 6)

Florida Keys Environmental Coalition stated that:

> "Why does Oxitec design contracts that require all data or public communications be controlled by Oxitec and each person participating in an experiment sign a long-term NDA? The EPA hasn't a pray of getting actual honest data from the field experiment in the Keys." (0400 p. 7)

The Institute for Pesticide Reform stated that:

> " . . . no amount of outrageous activity, not even the jailing of Industrial Bio-Test executives—the lab that defrauded the EPA and FDA by conducting bogus safety tests for chemical and pharmaceutical companies—has dislodged the agency's reliance on company data." (0461 p. 3)

Comment 0460 representing Mass Mailer, Penelope McMillan, Bobbie Flowers, Karen Barranco, John Ulloth, Jerilee Newby and Bruce Campbell stated that:

> "It's time for EPA to put away the rubber stamp and stop putting corporate profits before environmental protection." (0405 p. 1; 0411 p. 1; 0412 p. 2; 0413 p. 2; 0451 p. 2; 0454 p. 1; 0460 p. 2)

Maggie Mistal stated:

> "Please take a serious look at this and not just what the Oxitech [sic] or Mosquito Control tells you as they have much money to make off of this product but none of the accountability for

> damaging the environment. We are relying you [sic] in the EPA to protect the environment." (0408 p. 1)

EPA Response to Comments in Unit X.A. - Comments Requesting That EPA Extend the Comment Period. EPA determined it will not extend the comment period because the major thrust of the requests for an extension were in general coupled with a request for additional information/data. EPA concluded that an extension of the comment period was unlikely to meet the commenters requests for additional information because the Agency had supplied in the August 31, 2021 Notice of Receipt the information prescribed by regulation. The regulations at 40 CFR 172.11(a) require that certain types of information be provided to the public in the Notice of Receipt, i.e., the active ingredients, the use patterns, the quantity of pesticide to be used, the total acreage treated and the location of the area of application. That information was provided in the August 31, 2021 Notice of Receipt. The regulatory requirement at 40 CFR 172.11(a)(5) that the NOR provide the "Location or area of application" is satisfied by naming the State (e.g., "California") in which the application is to be made. The Agency is not required by 40 CFR 172.11(a) to supply in the Notice of Receipt any information beyond that specified in 40 CFR 172.11(a) such as any new information or data submitted by Oxitec to support its request for an extension and amendment of its existing EUP, or the Agency's analysis of that information.

EPA has included in the docket EPA-HQ-OPP-2019-0274, created for Oxitec's 2019 OX5034 EUP submission request, the Agency's analysis of Oxitec's 2019 submission as well as a document responding to public comment on Oxitec's original EUP request. The documents detailing EPA's analysis of the 2019 submission include the Human Health and Environmental Risk Assessment (0359), EPA's review of the details of the proposed experimental program (Review of Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 Aedes aegypti Mosquitoes (0352, 0356)), and the Memorandum on Vectorial Capacity (0351). These can be found in the docket EPA-HQ-OPP-2019-0274. The document (0355) responding to public comment on Oxitec's original 2019 EUP request can also be found in that docket.

EPA Response to Comments in Unit X.B. - 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 to the extent allowed by the statutes it administers. Some of the commenters requesting greater transparency requested that EPA's in-depth technical analysis of the OX5034 request for an EUP be made available to the public. EPA's analysis of Oxitec's 2019 application for an EUP to permit limited testing of OX5034 is part of the docket EPA-HQ-OPP-2019-0274 as well as its analysis of the 2021 Oxitec application to amend and extend the 2020 EUP, i.e., EPA's review of the details of the proposed experimental program (Review of Section G for an Experimental Use Permit 93167-EUP-2 to Test OX5034 Aedes aegypti Mosquitoes) and the Human Health and Environmental Risk Assessment. EPA also included the 2020 Response to Comment (0355) as well as this Response to Comments document in the docket (EPA-HQ-OPP-2019-0274).

With regard to comments on how the FIFRA CBI provisions affects transparency, FIFRA section 10 provides applicants with the ability to claim information as confidential, and requires EPA to protect information, which, in the Administrator's judgment, is entitled to confidential treatment. Conversely, FIFRA section 10(d)(1) also provides that safety and efficacy information must be made available to the public. The exceptions to this mandatory disclosure requirement are listed in FIFRA section 10(d)(1)(A), (B), and (C). The procedures for asserting confidentiality claims for safety and efficacy information are described in 40 CFR 158.33, 40 CFR 161.33, and PR Notice 2011-3. FIFRA section 10(g) prohibits the disclosure of certain information submitted by an applicant or registrant to any representative of a multinational pesticide producer or to anybody who intends to deliver such information to a multinational pesticide producer. Those persons may see certain abstracted data, or may view unabstracted data without copying. FIFRA provides that, in certain circumstances, the EPA Administrator may disclose information that is otherwise protected. Such action is rare, and is described in FIFRA sections 10(b), 10(d)(3), 10(g), and 12(a)(2)(D).

With regard to the comment that EPA did not provide the public the "full" advice of the US Centers for Disease Control and Prevention (CDC), EPA provided the public that advice in the document 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*" (EPA-HQ-OPP-2019-0274-0351). The document on Vectorial Capacity was developed in concert with experts at the CDC. These experts signed the document in confirmation of the conclusions reached on the issues analyzed in the document, including the analysis of the 2019 publication by Evans et al.,[28] on the potential for genetic material of Oxitec's *Ae. aegypti* OX513A mosquito to introgress into wild *Ae. aegypti* populations.

With regard to the comments that EPA did not provide information on the counties in which testing of OX5034 might occur, as noted in responses to comments found in Unit X.A of this Response to Comment document, the regulations at 40 CFR 172.11(a) require that certain types of information be provided to the public in the Notice of Receipt. The regulatory requirement at 40 CFR 172.11(a)(5) that the NOR provide the "Location or area of application" is satisfied by naming the State (e.g., "California") in which the application is to be made.

With regards to comments that the comparative efficacy of Wolbachia-infected mosquitos must be provided, please see the following section "EPA Responses to Comments in Unit IX.C. — Comments Arguing for Alternative Approaches" of the Response to Comments document associated with the original EUP (EPA-HQ-OPP-2019-0274-0355).

[28] Evans, B. R., P. Kotsakiozi, A. L. Costa-da-Silva, R. S. Ioshino, L. Garziera, M. C. Pedrosa, A. Malavasi, J. F. Virginio, M. L. Capurro, and J. R. Powell. 2019. Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population. Scientific Reports 9.

With regard to the comment that EPA did not provide to the public the results of testing performed by Oxitec in 2020-2021 under EUP 93167-EUP-E, EPA notes the original EUP is ongoing. Additionally, EPA does not routinely supply data developed during EUP testing to the public. The purpose of an EUP is to generate data to support registration of the pesticide product. Therefore, data from the EUP are typically submitted to the Agency with the application for registration of the product, and the Agency evaluates the data in determining whether the product meets the standard set by FIFRA for registration. With regards to the comment that some considerations for OX513A are applicable to the registration of OX5034, "EPA Response to Unit X. A. — Comments Expressing the Opinion that Insufficient Information Has Been Provided to the Public." of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274-0355).

With regards to there being no discussion of how OX5034 can be contained, see the Section G titled, "Review of the Section G (dated February 18, 2022) for an Experimental Use Permit (EUP) 93167-EUP-2 to Test OX5034 *Aedes aegypti* Mosquitoes in California and Extend the Existing EUP in Florida" that is included in the docket (EPA-HQ-OPP-2019-0274) for containment discussion. Additionally, more information can be found in the following section, "EPA Response to Unit XII.H. – Comments that EPA Should Ensure Post-Release Control Measures." of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274-0355). If at any time a female OX5034 mosquito is found, Oxitec, Ltd., must immediately cease releases of all OX5034 mosquitoes, as soon as practicable apply adulticide and larvicide pesticides to the treated area where the surviving females were detected, and continue to monitor for the presence of the OX5034 genetic construct in female *Ae. aegypti* until OX5034 mosquitoes are no longer found for at least two successive mosquito generations, a minimum of 10 weeks. Additionally, regardless of finding a female OX5034 mosquito, Oxitec, Ltd., must continue monitoring for a minimum of 10 weeks following the last observed fluorescent larvae in any release. For comments regarding how OX5034 mosquitos will be labeled, the label has been posted to the docket, EPA-HQ-OPP-2019-0274, at the time of permit issuance for the previous EUP as well as the amendment and extension.

EPA Response to Comments in Unit X.C. - Comments that EPA Should Seek Advice from Independent Committees of Experts. EPA sought advice and collaborated with experts at the Center for Disease Control while evaluating the original EUP application for OX5034 and with the California Department of Pesticide Regulation and Florida Department of Agriculture and Consumer Services while evaluating the amendment application. EPA did not seek the advice from its Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP).

With regard to the recommendation that the FIFRA SAP should be used to review Environmental Impact Statements (EIS) that commenters argue should be performed by EPA under NEPA for these products, the courts have consistently held that Congress did not intend for NEPA's requirements to apply to FIFRA's scheme for registering pesticides. Given the several judicial decisions regarding the relationship between FIFRA and NEPA, EPA does not perform an EIS in its regulation of pesticides. Unit X.H of this

Response to Comments document describes in greater detail the court decisions that form the basis of EPA's position on the relationship between FIFRA and NEPA.

EPA Response to Comments in Unit X.D. - Comments Arguing that EPA Should Seek Public Input. Referendums allow citizens to vote directly on an issue or proposition 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 application to amend and extend the EUP 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. 86 Fed. Reg. 48,691 (August 31, 2021). 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.

With regard to the comment that EPA should convene public meetings to allow the public to review the company's proposal and an EIS that commenters believe EPA should perform under NEPA, EPA has provided a number of documents in docket EPA-HQ-OPP-2019-0274 that provide the public information on Oxitec's OX5034 such as the human health and environmental risk assessment and the section G experimental protocol and review.

As noted at Unit X.C. and discussed in greater detail in Unit X.I, the courts have consistently held that Congress did not intend for NEPA's requirements to apply to FIFRA's scheme for registering pesticides. Given the several judicial decisions regarding the relationship between FIFRA and NEPA, EPA does not perform an EIS in its regulation of pesticides. Again, Unit X.I of this Response to Comments document describes in greater detail the court decisions that form the basis of EPA's position on the relationship between FIFRA and NEPA.

EPA Response to Comments in Unit X.E. - Comments Arguing That EPA Needs to Develop New Regulations for GE Insects and Animals. 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 and animals. The entirety of section 5 of FIFRA, 7 U.S.C. § 136c, was enacted by Congress to address experimental use permits, and EPA has promulgated regulations under that authority at 40 CFR part 172. For example, 40 CFR § 172.10 states that: "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." (Emphasis added).

EPA Response to Comments in Unit X.F. - Comment that EPA Should Ensure Post-Release Control Measures. EPA and its state partners can and do conduct inspections of EUP testing sites. With regard to comments concerning the role of other agencies, the US Federal Government's Coordinated Framework for Regulation of Biotechnology[29] describes the roles played by the various Federal agencies responsible for regulating the testing and commercialization of biotechnology products, including the role played by EPA. EPA, as a general matter, as it regulates a pesticide product can access the expertise of agencies such as the CDC, the FWS, National Marine Fisheries Service, National Invasive Species Council, and other US Federal agencies. The EPA does not administer or implement authorities assigned by Congress to the Center for Disease Control, or to any other Federal agency. The request from Oxitec for an amendment and extension of an EUP is being made under EPA-administered FIFRA. It remains Oxitec's responsibility to maintain compliance with its FIFRA responsibilities and with its responsibilities to maintain compliance with any other statutory authorities.

With regards to post-trial monitoring, see the Section G entitled, "Review of the Section G (dated February 18, 2022) for an Experimental Use Permit (EUP) 93167-EUP-2 to Test OX5034 *Aedes aegypti* Mosquitoes in California and Extend the Existing EUP in Florida" that is included in the docket (EPA-HQ-OPP-2019-0274) for discussion on monitoring. Additionally, more information can be found in Units VIII.A and VIII.B of this Response to Comment document on the monitoring conditions EPA has placed on the trial.

EPA Response to Unit X.G. - Comments Arguing that EPA Must Address Human Testing. 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 part 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.

[29] https://www.epa.gov/sites/default/files/2017-01/documents/2017_coordinated_framework_update.pdf

Several comments suggested the ethical standards that should be applied to this research and asserted that informed consent of those living in the area of the Oxitec release must be obtained prior to initiating the research. 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 governing the ethical conduct of the research under this EUP are found in EPA's Rule for the Protection of Human Subjects of Research (40 CFR part 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 part 26, Subparts K-L:

> 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."
>
> 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))

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 amended and extended EUP, Oxitec would collect information on how efficacious releases of male OX5034 mosquito are at suppressing wild *Ae. aegypti* mosquito populations in the test areas. 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 determine the over-flooding ratio (i.e., the OX5034 male to wild male ratio) and the 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" (dated February 18, 2022) for an Experimental Use Permit (EUP) 93167-EUP-2 to Test OX5034 *Aedes aegypti* Mosquitoes in California and Extend the Existing EUP in Florida" that is included in the docket (EPA-HQ-OPP-2019-0274)

As described earlier in Unit IV of this Response to Comment document, during testing, only male mosquitoes will be released. The 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. Oxitec is not proposing to collect any information about individuals in the area of the release or to monitor behavior of individuals or their interactions with mosquitoes. 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 OX5034 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 part 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 Comments in Unit X.H. - Comments Addressing Other Ethical Issues. With regard to environmental justice considerations, EPA is fully committed to environmental justice as described in Executive Order 12898. EPA works to integrate the Agency's consideration for communities with environmental justice concerns into the Agency's policy decisions. With regard to OX5034, commenters who voice the opinion that OX5034 might harm communities with environmental justice concerns do not offer explanations on how OX5034 might harm such communities in general, nor do they offer any explanation of how OX5034 might cause harm during EUP testing. EPA has carefully assessed OX5034 and found the probability of harm to human health and the environment from testing of OX5034 under the EUP issued in 2020, and again when evaluating the request to amend and extend the EUP in 2022.

With regard to comments with ethical concerns about interfering with the population of a species and raising the possibility of extinction, the pesticidal effect of OX5034 *Ae. aegypti* males requires continuous releases and is time limited. As described in the 2020 Human Health and Environmental Risk Assessment in Unit II.C "Environmental Fate of the Transgenes and OX5034 Background Genetics" (EPA-HQ-OPP-2019-0274-0359), the transgene will not persist in the environment and is expected to disappear within 10 mosquito generations once releases have ceased. The self-limiting nature of OX5034 *Ae. aegypti* limits OX5034 *Ae. aegypti* both spatially and temporally. The local *Ae. aegypti* population is expected to rebound once OX5034 *Ae. aegypti* releases have ceased and therefore the releases will not result in the extinction of a species.

With regard to the comment expressing concern about reducing any insect population in light of the general decline of insect populations, as described in the 2020 Human Health and Environmental Risk Assessment in Unit II.D "Environmental Effects Assessment" (EPA-HQ-OPP-2019-0274-0359) and reaffirmed in the 2022 Human Health and Environmental Risk Assessment in Unit III.C "Environmental Effects Assessment," *Ae. aegypti* do not play a critical role in the diet of any predators, including any insect predators, and no non-target organisms (including other insects) are expected to be impacted should the local *Ae. aegypti* population be suppressed. Additionally, *Ae. aegypti* is a major pest species with known impacts on human health by vectoring disease and is already continually suppressed by other control methods such as the use of chemical and microbial insecticides as well as breeding site source reduction.

With regard to the comment that EPA should employ a precautionary approach under FIFRA, EPA carefully evaluated OX5034 to determine whether testing might present risk of harm to human health or the environment and found the probability of harm to human health and the environment from testing of OX5034 under the EUP issued in 2020, and again when evaluating the request to amend and extend the EUP in 2022, to be negligible. See the Agency's 2020 and 2022 Human Health and Environmental Risk Assessments in the docket for this action (EPA-HQ-OPP-2019-0274).

EPA Response to Comments in Unit X.I. - Comments Arguing that EPA Must Prepare a Full EIS Under the National Environmental Policy Act (NEPA). Some comments assert that although "proposed actions under FIFRA have been exempt from NEPA," … an assessment under NEPA should be required because "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."

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.

In *Environmental Defense Fund, et al. v. Environmental Protection Agency, et al.*, 489 F.2d 1247 (D.C. Cir. 1973), the D.C. Circuit 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 1256. 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*, 747 F.3d 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 discusses its responsibilities with regard to OX5034 under the EPA Response to comments addressing the Endangered Species Act in Unit X.J. – "Comments Regarding the Endangered Species Act."

EPA Response to Comments in Unit X.J. - Comments Regarding the Endangered Species Act. Regarding comments that EPA must comply with the ESA and perform an endangered species assessment for the EUP, EPA previously performed an endangered species assessment for the original EUP issued in April 2020 and has completed a new assessment for the application to amend and extend the EUP (93167-EUP-2). The previous endangered species assessment can be found in the Human Health and Environmental Risk Assessment prepared in 2020 for the original EUP request in the docket (EPA-HQ-OPP-2019-0274-0359) with the analysis for nontarget organisms under Unit II.D. A ''No Effect'' determination was made for direct and indirect effects to federally listed endangered and threatened species, and for their designated critical habitats based on the likelihood of low exposure and no anticipated effects from consumption of OX5034 mosquitoes or the suppression of the local *Ae. aegypti* population. The endangered species assessment for the current application to amend and extend Oxitec's EUP can be found in the Human Health and Environmental Risk Assessment prepared in 2022 (see Units III.C, V, and the Appendices). EPA similarly concluded that there is a reasonable expectation of no discernible effects for nontarget organisms as a result of the proposed experimental use permit extension and amendment to release OX5034 mosquitoes. Therefore, since no discernable effects are anticipated to any nontarget organism, a ''No Effect'' determination was also made for direct and indirect effects to federally listed endangered and threatened species, and for their designated critical habitats.

Regarding comments that EPA should have consulted with the U.S. Fish and Wildlife Service and/or other wildlife experts, EPA concluded that the original EUP (93167-EUP-) and the current amendment and extension (93167-EUP-2) would have "No Effect" on federally listed endangered and threatened species or their designated critical habitats. EPA is therefore under no obligation to consult with the Services, because as noted by comment (0396), consultation is required only when an agency determines that the action "May Affect" a listed species or critical habitat ("informal consultation" if EPA had made any "May Affect – Not Likely to Adversely Affect" determinations, and "formal consultation" if EPA had made any "May Affect – Likely to Adversely Affect" determinations).

Regarding comments stating that no additional analyses were performed for the state of California, this is incorrect. At the time of the publication of the Notice of Receipt, the risk assessment for the application to amend and extend Oxitec's EUP, including the endangered species assessment, were still ongoing as is standard practice. However, in addition to its consideration of whether the application to amend and extend the EUP met the FIFRA standard for issuance undertaken prior to issuance of the EUP amendment, EPA performed an ESA assessment (see Units III.C, V, and the Appendices).

Regarding comments questioning which locations and therefore which species should be included in the ESA assessment, EPA included in its ESA assessment all federally listed threatened and endangered species present in the counties selected for proposed testing. For increased transparency, EPA has included a list of the federally listed species present in the counties for proposed testing in the Appendix of the Human Health and Environmental Risk Assessment prepared in 2022), which is located in the docket established for this action.

EPA Response to Comments in Unit X.K. - 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 Comments in Unit X.L. - Comments on Economic Considerations. With regards to the cost of OX5034 *Ae. aegypti* mosquitoes, particularly in comparison to other mosquito control methods, please see "EPA Responses to Comments in Unit IX.C. – Comments Arguing for Alternative Approaches" of the Response to Comments document associated with the original 2020 EUP (EPA-HQ-OPP-2019-0274-0355). In Unit III of this Response to Comment document, representatives of Mosquito Control Boards describe the role these Boards see for OX5034, or similar mosquito products, in their future planning for contingencies such as the loss of mosquito control tools through the growing emergence of resistance to chemical pesticides in mosquito populations.

With regard to comments arguing for more testing based on concerns about potential of OX5034 to affect tourism, EPA evaluated for risk releases of OX5034 mosquito in its Human and Environmental Risk Assessment prior to issuing the EUP in April 2020 (EPA-HQ-OPP-2019-0274-0359) and again in 2022 when evaluating the application to amend and extend the EUP (also in docket EPA-HQ-OPP-2019-0274) and determined that there would be no adverse effects to humans or the environment as a result of the experimental use permit to release Oxitec's OX5034 male mosquito. The risk assessments carefully evaluated whether female OX5034 females might occur in the test areas or might move out of the test areas. These concerns have also been addressed in the 2020 Response to Comment and again in this current Response to Comment document (see Units IV and VIII).

With regard to comments arguing for more public outreach based on concerns about potential of OX5034 to affect tourism, EPA has addressed comments requesting opportunities for more public input in Unit X.D.3 of this Response to Comment document.

With regards to the comment arguing an analogy between OX5034 and trade consequences arising in food commodities containing unapproved genetically engineered events, the OX5034 mosquitos are not part of a traded physical commodity.

With regards to the scenario where tourism may be impacted by OX5034 *Ae. aegypti* mosquito releases, the commenters did not provide evidence that a significant portion of people who travel to these areas for leisure are concerned about genetically modified mosquitoes, and therefore EPA is unable to respond further.

With regards to the types or amount of liability insurance a private company holds, this is outside the scope of the EUP. EPA does note, however, that the hypothetical scenarios raised by the commenter have previously been addressed. Regarding mosquitoes moving out of the test area, please see "EPA Response to Unit X.D. – Comments on the Potential for Mosquitoes to Move Away from the Test Area" of the Response to Comments document associated with the original EUP (EPA-HQ-OPP-2019-0274-0355). Regarding the potential for female OX5034 mosquitoes to be present in the test areas or post-testing, please see "EPA Response to Unit V.B – Exposure considerations" and "EPA response Unit V.C. – Hazard considerations" of this Response to Comments document.

EPA Response to Comments in Unit X.M. - Comments on Disease Incidence and Recommendations that Oxitec Apply to FDA. With regard to comment questioning why Oxitec is not, under the EPA issued EUP, required to test to determine whether OX5034 would reduce disease transmission, the OX5034 mosquitoes are intended for suppression of *Ae. aegypti* mosquito populations. Therefore, studies conducted under the EUP that are required to support this product for registration under FIFRA section 3 center around monitoring for effects on *Ae. aegypti* mosquito population size.

With regard to the comment that Oxitec should be required to contact FDA about OX5034, the EPA does not administer or implement Federal Food, Drug and Cosmetic Act (FFDCA) authorities assigned by Congress to the Food and Drug Administration. The request for an amendment and extension of an EUP is being made under EPA-administered FIFRA. It remains Oxitec's responsibility to maintain compliance with any other statutory authorities.

With regard to comments that the incidence of diseases vectored by *Ae. aegypti* is low in the US, EPA refers the reader to Unit III.B. of this Response to Comment document for additional details on the incidence of diseases vectored by *Ae. aegypti* in the US. Also discussed are concerns that these arboviruses are currently circulating in the Caribbean and Mexico and thus could enter the US at any time.

EPA Response to Comments in Unit X.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 and assure the quality and integrity of data submitted. EPA regularly audit laboratories to ensure the quality and integrity of test data submitted to the Agency under Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

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.[30,31] 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

[30] 40 CFR Part 158.2080 - Experimental use permit data requirements – biochemical pesticides.
[31] Memorandum: R. McNally to M. Mendelsohn and S. Borges. BPPD Guidance for Senior Staff and Branch Chief Review of Guidance Documents. August 14, 2018.

published studies in peer reviewed scientific journals.[32] 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.

[32] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-identifying-selecting-and-evaluating-open (last accessed: 02.18.2022)

Appendix

Number	Commenter	Organization
0344	Jaydee Hansen	Center for Food Safety; received on 10/11/2019, comment incorporated by reference by 0396
0365-1	Truc Dever	Mosquito and Vector Control Association of California (MVCAC)
0365-2	Ryan Clausnitzer Jeremy Wittie Steve Mulligan Mustapha Debboun J. Wakoli Wekesa Truc Dever Rick Howard Gary Goodman Peter Bonkrude Michelle Brown	Alameda County Mosquito Abatement District Coachella Valley Mosquito and Vector Control District Consolidated Mosquito Abatement District Delta Mosquito and Vector Control District East Side Mosquito Abatement District Greater Los Angeles County Vector Control District Orange County Mosquito and Vector Control District Sacramento-Yolo Mosquito and Vector Control District Shasta Mosquito and Vector Control District West Valley Mosquito and Vector Control District
0366	Anonymous	
0367	Mustapha Debboun	Delta Mosquito & Vector Control District, Visalia CA
0368	Dylan Russell	
0369	Dolores Marchese	
0370	Anonymous	
0371	Edward Russo	
0372	Edward Russo	
0373	Jo Rand	
0374	Phil Goodman	Florida Keys Mosquito Control District
0375	Norman C. Leppla	University of California
0376	Kathryn Woodfine	
0377	Julian Morris	Reason Foundation
0378	Samantha Killheffer	
0379	Tim Gordon	
0380	Frances Miklem	
0381	David Brown	American Mosquito Control Association
0382	Jaydee Hansen	Center for Food Safety
0383		GeneWatch UK
0384	Mary Hollowell	
0385	Christopher Lesser	Florida Mosquito Control Association Florida Mosquito Control Foundation
0386	Mark van der List	
0387		Listed as Oxitec but actually GMO Free Florida
0388	Christopher Lish	
0389	David Brown	American Mosquito Control Association
0390	Clint Nesbitt	BIO
0391		ADAPCO, LLC
0392	Dan Killingsworth	Environmental Security Pest Control
0393		American Mosquito Control Association

		Anastasia Mosquito Control District Delta Mosquito and Vector Control District Entomological Society of America Mosquito and Vector Control Association CA Society for Vector Ecology Northwest Mosquito and Vector Control Assn.
0394	Dana Perls	Friends of the Earth
0395	Lori Tremmel Freeman	
0396	Jaydee Hanson Jenny Loda	Center for Food Safety
0397		GMO Free Florida
0398	Lori Tremmel Freeman	National Association of County and City Health Officials
0399	Meagan Morrison Hull	
0400	Barry Wray	Florida Keys Environmental Coalition
0401	Sarah C. Aird	Californians for Pesticide Reform
0402	Lisa Ann, Chad, George, Geoffrey, &Tristan Kelly	
0403		Marian Wilson Butterfly Garden
0404	Andy Yoken	
0405	Penelope McMillan	
0406	Anonymous	
0407	Robert Marquardt	
0408	Maggie Mistal	
0409	Jessica Denning	
0410	Julie Ostoich	
0411	Bobbie Flowers	
0412	Karen Barranco	
0413	John Ulloth	
0414	Edard Russo	
0415	Leor Kaminski	
0416	Derek Trial	
0417	Anonymous	
0418	Jerry Russell	
0419	Anonymous	
0420	Mary Hollowell	
0421	Lawrence Hribar	
0422	Andrea Leal	
0423	Tekia Fiorentino	
0424	Jan Wang	
0425	Taia Pinholster	
0426	Kimberly Sikora	
0427	James Norrie	
0428	Casey Schlinker	
0429	Anonymous	
0430	Judith Borcz	
0431	Anonymous	

0432	Andy Peri	
0433	K. Bluefield	
0434	Andria Ventura	
0435	Adam Continenza	
0436	Deborah Ramelli	
0437	Anonymous	
0438	Shelly Ready	
0439	Anonymous	
0440	Michael Burton	
0441	Pamm Larry	
0442	Susan Rigalli	
0443	Laura Moreno	
0444	Susan Brierly	
0445	Robert Gregory	
0446	Keith Schildt	
0447	Deanna Figueira	
0448	Susan Warren	
0449	Joni Stellar	
0450	Ariane Glazer	
0451	Bruce Campbell	
0452	Craig Zabransky	
0453	Belinda Mostert	
0454	Jerilee Newby	
0455	Linda Delair	
0456	Jessica Denning	
0457	Anonymous	
0458		Florida Keys Environmental Coalition
0459	Michael Weissman	
0460	3,852 comments	Representing Mass Mailer
0461	3,336 signatures	Institute for Responsible Technology
0462	4,258 signatures	Friends of the Earth
0463	313 signatures	Center for Food Safety
0464	1,106 signatures	Center for Food Safety