To: Food and Drug Administration
Docket Number: FDA-2014-N-2235-1005
Regarding: Draft Environmental Assessment and Preliminary Finding of No Significant Impact Concerning Investigational Use of Oxitec OX513A Mosquitoes; Extension of Comment Period
From: Effective Altruism Policy Analytics

The FDA's Preliminary Finding of No Significant Impact relates to a proposed trial of Oxitec's OX513A engineered strain of *Aedes aegypti* mosquitoes in Key Haven, Florida. We found the environmental assessment prepared by Oxitec to be comprehensive and agree with the FDA's finding that running the trial will have no significant negative impacts on the quality of the environment or human health.

The release of male OX513A mosquitoes allows *Ae. aegypti* populations to be suppressed in a form of Sterile Insect Technique (SIT) known as Release of Insects carrying a Dominant Lethal (RIDL). The OX513A genome contains a dominant tetracycline-dependent transcriptional activator, tTAV, that causes overproduction of protein and kills more than 95% of the mosquito's progeny before they reach adulthood. Although it is not necessary for the RIDL technique, OX513A also contains a red fluorescent marker, DsRed2, that allows the mosquitoes to be tracked.

We were initially concerned about environmental and health risks associated with the trial, but are confident that they are **not significant**. We have identified a number of benefits related to the RIDL technology and believe this trial will help develop this technology. **We thus urge the FDA to continue with its Finding of No Significant Impact**. We summarize our research on the risks and benefits of the trial below:

Potential Risks

Few Surviving Progeny: We do not think there is a risk that a significant OX513A *Ae. aegypti* population will persist in the environment after this trial. In the absence of tetracycline, only 3–4% of OX513A mosquitoes survive to adulthood (Phuc et al. 2007). In the field, we would expect even lower survival rates. In fact, during a field release on Grand Cayman Island (Harris et al., 2007), eggs with the fluorescent marker were recovered up to a week after release of OX513A males, but not after, suggesting that no OX513A mosquitoes survived to the second generation.

Few Resistant Progeny: The small number (less than 4%) of surviving OX513A progeny may survive due to resistance to the lethal effect of tTAV and pass that resistance onto their own progeny. Even if this is the case, previous field trials found that OX513A are less able to compete for mates than their wild-type counterparts (Carvalho et al., 2015; Harris et al., 2007). After cessation of the trial we would expect the OX513A population to be outcompeted by wild-type *Ae. aegypti* migrating from outside the trial area. We expect that the only way an

OX513A population can be maintained is via the sustained weekly release programs common in SIT.

Environmental tetracycline exposure is unlikely to allow survival: While greater than 4% of OX513A progeny are able to survive when exposed to tetracycline (at a concentration of >1 ng/mL), we believe there is very little risk that mosquitoes released in this trial will encounter sufficient tetracycline. Concentrations of environmental tetracycline in the ng/mL range are only found in effluents from hospitals and commercial farms (Homem & Santos, 2011); there are no commercial farming operations in Key Haven and the nearest hospital/clinic is well over 300 meters away from the release site. That distance is greater than the largest mean dispersal distance reported for any type of *Ae. aegypti* travel in their preferred habitat.[1] All of the testing area north to the intersection of Key Haven Road and Driftwood Drive is out of range for record Ae. agypti dispersal distances. Given the additional water barrier, it is unlikely females will be able to lay eggs in areas with higher tetracycline levels. Similarly, human blood after a course of tetracycline antibiotics does not contain a sufficient concentration to improve survival, as confirmed by the study in Appendix G of Oxitec's Environmental Assessment.

Control with Pesticides: In the unlikely event that an OX513A population should persist in the area after the trial, the Florida Keys Mosquito Control District (FKMCD) expects to be able to track and eliminate them using traditional pesticide control (FKCMD, 2016). The DsRed2 fluorescent marker present in the OX513A genome allows both released OX513A adults and their progeny to be easily tracked.

No Horizontal Gene Transfer: There is very little risk of horizontal gene transfer between OX513A and other species. Even though there are, on occasion, cross-species insemination events between *Ae. aegypti* and the closely-related *Ae. albopictus* in the field (Tripet et al., 2011), no viable progeny have been observed either in the field study or in forced matings (Nazni et al. 2009). We do not expect Oxitec's genetic modifications to be spread sexually to other mosquito species.

No Risks to Mosquito Predators: The trial's temporary suppression of the *Ae. aegypti* population of Key Haven should not adversely affect predator species; *Ae. aegypti* is a non-native species in North America and is generally found in and around human habitation, making up only a small percentage of the diet of its largely-opportunistic predators (Murphy et al., 2010, Draft Environmental Assessment Section 13.5.2). For the predators that do opportunistically consume OX513A mosquitoes, there is very little risk of toxicity from the small amounts of dietary DsRed2 or tTAV they would encounter as a result, as confirmed by a toxicity study in which two *Ae. aegypti* predators were fed OX513A larvae as 70-100% of their diets and showed no adverse effects (Appendix H).

[1] A summary of literature on mean *Ae. aegypti* dispersal distances is provided in Figure 17 of Oxitec's Draft Environmental Assessment

OX513A Bites No Worse For Human Health: The risk of exposure to the novel proteins from mosquito bites is low—no evidence of tTAV or DsRed2 protein could be detected in the saliva of female OX513A mosquitoes (Appendix K). The low overlap of the mosquito and human genomes (*cf.* Neafsey et al. 2016) strongly suggests that mosquito bites do not cause a transfer of genomic DNA into human cells. In addition, the risk of any biting events occurring from OX513A mosquitoes is relatively low, as very few adult female OX513A mosquitoes are expected to be released (at most 0.03% of the total, following Carvalho et al. 2015) and less than 5% of progeny survive.

No Significant Tetracycline Release from Rearing: Aside from the risks to human health from mosquito bites, which we believe to be insignificant, there might be risks associated with the rearing facility in Marathon, FL, especially the use of the antibiotic tetracycline (Curtis et al. 2015). However, the concentrations (30 ug/mL before any dilution, according to Curtis et al., 2015) of tetracycline used in rearing are lower than in many existing applications. For example, commercial swine farming operations sometimes release as much as 200 mg/L tetracycline in manure (Kumar et al. 2005). Development of antibiotic resistant bacteria in the guts of OX513A larvae is not of concern, since mosquito gut bacteria are lost during metamorphosis into adults (Moll et al. 2001).

Unlikely to develop new characteristics via mutation: Other comments (e.g. FDA-2014-N-2235-1284) have expressed concern about the *piggyBac* transposon ("jumping gene") used to add the tTAV and DsRed2 genes to OX513A. Although the *piggyBac* transposon is able to integrate into the *Ae. aegypti* genome, the integrated element does not later jump around to other locations; the unusually high stability of *piggyBac* in *Ae. aegypti* has in fact been the subject of scientific study (Arensburger et al. 2011, Sethuraman et al. 2007). Sequencing the genomes of OX513A mosquitoes by Oxitec showed genetic stability across more than 36 generations.

***Ae. aegypti* unlikely to be replaced by *Ae. albopictus*:** If the population of *Ae. aegypti* is reduced, there is concern that its niche might be filled by another, possibly more dangerous, mosquito species such as *Ae. albopictus*. A review of the vector status of Aedes albopictus by Gratz (2004) indicated that *Ae. aegypti* is more frequent on the coasts and at low altitude, while *Ae. albopictus* is more plentiful inland and at higher altitudes.The Florida Keys is a habitat much more suited to *Ae. aegypti* than it is for *Ae. albopictus* and replacement is unlikely.

Potential of Psychological Harm: The release of OX513A is likely to cause some psychological harms due to fear of the genetically-modified mosquitoes. We believe this fear is not well-founded, but likely. We created a model to estimate the the impact on the residents of Key Haven and the found that there could be a loss of around 1 Quality-Adjusted Life Year (QALY) (90% confidence interval: 0.073 to 5.2) across the population.[2] Another model we estimated that the experience of less preferred vacations due to fear during the trial might lead

[2] Estimates generated by Monte Carlo Simulation may change slightly as the models are reloaded.

to a net loss of about 1 QALY across the large number of vacationers during the trial. We advise Oxitec to devote resources to properly educating the citizens of Florida Keys in the general safety of OX513A mosquitoes.

Potential Benefits

Mosquito control using Oxitec's OX513A strain is likely to result in significant public health benefits for the United States as well as the rest of the world, and we believe that the benefits of holding a trial far outweigh the risks. The primary benefit of approving this field trial would be to speed up the adoption of OX513A for mosquito control in the United States.

Release of OX513A mosquitoes has been shown to reduce *Ae. aegypti* populations by up to 95%, bringing the number of pupae per person 10 times below the threshold needed for epidemic transmission of dengue fever (Carvalho et al, 2015). The high effectiveness of RIDL-based vector control suggests that accelerating the testing and deployment of OX513A could eliminate a substantial proportion of all the infections caused by *Ae. aegypti*.

The benefits of mosquito control with RIDL depend on the current burden of disease caused by *Ae. aegypti*. The potential benefits are large, as viruses transmitted by *Ae. aegypti* cause thousands to tens of thousands of infections annually in the United States, mostly of dengue fever (Dengue in Puerto Rico, 2015); in the rest of the world, where *Ae. aegypti* causes tens of thousands of annual deaths as well as a comparable burden of disease from non-fatal infections, the possible benefits are even greater.

Within the United States, Puerto Rico is most affected by diseases transmitted by *Aedes aegypti*, including dengue fever, Zika fever, and chikungunya. Although *Ae. aegypti* has limited impact elsewhere in the country, the number of mosquitoes is likely to increase due to climate change, and improved methods of mosquito control would help counteract this change.

In addition to direct health benefits, improved control of mosquito populations is likely to have considerable tourism benefits for Puerto Rico. Tourism comprises a significant portion of Puerto Rico's economy, contributing $2.4 billion to GDP in 2014 (2.4% of total GDP) (World Travel & Tourism Council, 2015). The CDC has issued a Level 2 travel warning for Puerto Rico due to local transmission of Zika virus ("Zika Virus in Puerto Rico", 2016), causing a fall in the share prices of travel companies (Margolis, 2016). The impact on tourism is likely to become worse if Zika spreads further.

Disease burdens

Ae. aegypti is responsible for a substantial burden of disease within the United States, so mosquito control with OX513A could bring considerable benefits. The disease burden is even more severe outside the United States, and this trial will guide improvements and deployment in

other disease-burdened areas. Our estimates of the burden of disease caused by *Ae. aegypti* are listed below (key numbers in **bold**):

Dengue fever: In the United States, dengue is primarily found in Puerto Rico. In 2010 alone, over **26,000** suspected cases of dengue were reported on the island ("Dengue in Puerto Rico", 2013) and of the cases tested in a laboratory, 47% tested positive. The annual costs of medical treatment and work absences resulting from dengue in Puerto Rico are estimated at approximately **$46 million** (Yalasa, Shepard & Zeng, 2012). A dengue epidemic in 2007 caused at least **11 deaths** (Tomashek et al., 2012). Worldwide, approximately **11,000 lives** and **1.14 million disability-adjusted life years** are lost due to dengue each year (Stanaway et al., 2016). Our own estimates, using more current WHO data, suggest a burden of **2.3 million disability-adjusted life-years** (90% confidence interval 1.5M-3.4M) per year.[3]

Zika fever: As of May 1, there have been 596 locally-acquired cases of Zika in the United States ("Zika virus in the United States", 2016). The most serious cases of Zika complications so far have mostly occurred in Brazil, where the Anthony Costello of the World Health Organization estimates that Zika infection has resulted in 2,500 cases of microcephaly ("WHO Virtual Press Conference on the Zika virus", 2016). Since Zika has only recently been recognized to cause microcephaly, the per-infection risk is unclear, and it is difficult to estimate how many cases of microcephaly will occur within the United States.

Yellow fever: Yellow fever is not endemic to the United States, but in the rest of the world it may cause more deaths than any other disease transmitted by *Aedes* mosquitoes—in 2013, **from 29,000 to 60,000** deaths in Africa alone ("Fact sheet: yellow fever", 2016). Using this, we created a model that estimates a total DALY burden of 1.3 million (90th percentile confidence interval: 890K to 1.8 million).

Chikungunya: Chikungunya has recently spread to Puerto Rico, where over 1,000 suspected cases were reported in 2015 ("Informe Semanal de Vigilancia de Chikungunya", 2016). Far larger outbreaks have occurred outside the United States; one of the largest outbreaks, occurring in 2006 in India, caused approximately 1.2 million infections ("Chikungunya in India", 2006) and the loss of ~25,000 disability-adjusted life years (Krishnamoorthy et al., 2009).

Comparison to other techniques

Compared to other strategies for suppressing mosquito populations and/or disease transmission, Oxitec's RIDL approach has significant advantages in safety and effectiveness.

Sterilization using radiation: Mosquito populations can be suppressed by releasing irradiated sterile males that mate with wild females and prevent their reproduction. Per released mosquito, this approach is likely to be less effective than releasing OX513A males, since the males are

[3] Estimates generated by Monte Carlo Simulation may change slightly as the models are reloaded.

harmed by irradiation and as a result are less able to compete with wild males. In the related mosquito *Aedes albopictus*, irradiated sterile males achieved a maximum "competitiveness index" of 0.53 compared to wild males (Oliva et al., 2012) in cage tests; on the other hand, OX513A males appear to have equal mating competitiveness as wild males in cages (Lee et al., 2012).

***Wolbachia* cytoplasmic incompatibility:** Another alternative to genetic modification for mosquito population suppression exploits *Wolbachia*-induced cytoplasmic incompatibility (CI). CI causes the offspring of infected and uninfected mosquitoes to die as embryos, so releasing infected males will suppress wild mosquito populations (Lees et al. 2015). A *Wolbachia* strain has been introduced into *Ae. aegypti* populations and shown to reduce dengue transmission (Hoffmann et al., 2011); this same strain also shows reduced transmission of chikungunya (van den Hurk et al, 2012). However, releasing *Wolbachia*-infected mosquitoes poses potential risks—there are concerns that *Wolbachia* from infected populations may spread to other species (Loreto and Wallau, 2016). In addition, while infecting a mosquito population with *Wolbachia* potentially irreversible, the effects of RIDL can be reversed simply by ending the release of genetically modified mosquitoes.

Gene drive: More recently, researchers have been developing "gene drive" systems where a gene biases its own inheritance to spread throughout a mosquito population. Using a gene drive to introduce genes that are lethal to mosquitoes, bias the sex ratio in favor of males, or reduce each mosquito's likelihood of carrying diseases could substantially reduce disease transmission. Although this approach appears promising, the technology is far from mature, and releases of gene drive systems will be more difficult to control and/or reverse than RIDL.

Responses to comments

Results of past trials (FDA-2014-N-2235-0026)

The commenter is concerned that too little is known about the results of previous releases of genetically modified mosquitoes. The results of past trials of the same strain of mosquitoes in the Cayman Islands (Harris et al., 2007) and Brazil (Carvalho et al., 2015) have been published and demonstrated the safety and effectiveness of Oxitec's approach. We believe the minimal risks of this trial are far outweighed by its potential to help reduce disease transmission in the United States and the rest of the world.

How many mosquitoes will be released? (FDA-2014-N-2235-0034)

The commenter, Cathy Sullivan, states:

> No actual or estimated physical number of genetically engineered/modified mosquitoes proposed to be released in the Florida Keys field trial is stated anywhere in Oxitec's 286 page Environmental Assessment as published... If it is to be tens of millions of GM

mosquitoes released, as was done in Brazil, more than 20,000 of these GM mosquitoes will be blood thirsty females seeking human hosts.

Current sorting techniques yield at most 0.03% females (Carvalho et al., 2015), so even if 10 million mosquitoes were released, fewer than 2,500 would be female, far below the 20,000 estimated by Ms. Sullivan. In fact, the number released should be far lower than Ms. Sullivan estimates: a successful trial of OX513A for mosquito suppression in Brazil (Carvalho et al., 2015) released fewer than 70,000 mosquitoes per week in an area with 1,800 residents. If 0.03% of the released mosquitoes were female, only 1 female was released per 60 residents per week.

A satellite image of Key Haven shows no more than 111 houses in the testing area (labeled "TA" in Figure 10 of Oxitec's Draft Environmental Assessment). If the number of mosquitoes released per house is equal to the number in the Itaberaba trial, we estimate that approximately 25,000 OX513A mosquitoes will be released, of which on the order of 10 will be female, too few to significantly increase disease transmission. The number of female mosquitoes released will be insignificant compared to the expected 85-95% reduction in mosquito populations.

Environmental release of tetracycline (FDA-2014-N-2235-0025)

As stated above in the "No Significant Tetracycline Release from Rearing" section, the concentrations of tetracycline used for rearing mosquitoes (30 ug/mL before any dilution, according to Curtis et al., 2015) is much lower than in many other applications; for example, swine farming operations can release as much as 200 mg/L tetracycline in manure (Kumar et al. 2005).

Harm to predators of mosquitoes (FDA-2014-N-2235-0029)

As we discuss in the "No Risks to Mosquito Predators" section, OX513A mosquitoes are unlikely to directly harm predators of *Ae. aegypti*, since the additional proteins present in the OX513A strain are not harmful or allergenic. Eradicating *Ae. aegypti* is also unlikely to deprive any predator species of a significant food source, as it is a non-native mosquito regularly targeted by mosquito control interventions (Oxitec Draft Environmental Assessment, p. 79).

Connection to Zika Virus (e.g. FDA-2014-N-2235-0026, FDA-2014-N-2235-0707, FDA-2014-N-2235-1255)

Many previous comments suggest that this trial is being carried out because of increasing concern in the United States about Zika virus. Zika virus is transmitted by *Aedes* mosquitoes such as *Ae. aegypti*, so its transmission could also be limited by OX513A release and subsequent population control of *Ae. aegypti*. However, the study proposed by Oxitec is not focused on Zika and the OX513A technology was developed in response to other diseases

spread by *Ae. aegypti*, such as Dengue fever, as Zika virus was not considered a serious health hazard before the recent outbreak of microcephaly (Malone et al. 2016, Butler 2016).

Conclusion

We find the environmental assessment prepared by Ozitec to be comprehensive and agree with the US Food and Drug Administration's Finding of No Significant Impact. We have analyzed both the potential environmental and public health risks that we initially considered, as well as potential dangers brought up from the other comments, and have concluded that all dangers are minimal or non-existent.

In addition, we have analyzed the potential benefits of this experiment and found them to be quite large, both to the US and to the rest of the world. Moreover, the Oxitec RIDL approach is safer and more effective than other proposed methods of suppressing mosquito populations and disease transmission. We believe that delaying further on this experiment will be an egregious error from a public health and humanitarian perspective. Thus we unequivocally support the test release of the Oxitec 513A Mosquitoes and urge the FDA to approve trials with all due haste.

Sincerely yours,

Matthew Gentzel, Co-founder Effective Altruism Policy Analytics
Tessa Alexanian, Automation Research Assistant at Zymergen
Linchuan Zhang, Student at Signal Data Science
Rachel Miller, Strain Build Research Assistant at Zymergen
Salvador Rivera, Director of Human Resources at Zymergen
Melissa Netwal, Genome Build Research Assistant at Zymergen
Dr. Matthew Davis, Scientist at Zymergen
Dr. Youngnyun Kim, Scientist at Zymergen
Harmen van Rossum, Scientist at Zymergen
Derek Shpiel, Research Assistant II at Zymergen
Matthew Youngblood, Analytical Chemist at Zymergen
Dr. Peter Enyeart, Scientist at Zymergen
Megan Crawford, B.S. in Entomology UC Davis
Dr. Jared Wenger (Scientist) at Zymergen
Eric Yu, Research Intern at Bolt Threads

Appendix A

Communication between Matthew Gentzel and John Min on the safety of OX513A

Hi Matthew,

You are correct that the OX513A mosquitoes from Oxitec do not employ any form of biased inheritance, and definitely do not use any form of gene drives. Rather, OX513A rely on Mendelian dominance. My understanding is that their genetic system relies heavily on the expression of the tTA gene, which will accumulate and kill a mosquito in the late larvae to pupae stage. The gene is strong enough that a single copy is sufficient to be lethal, hence it is dominantly inherited. Oxitec is able to raise these mosquito in their facilities by adding tetracycline to the water, which initiates a suppression system (that they designed and knocked into their mosquito) to prevent tTA accumulation. Since tetracycline would not be present in the environment, 100% of the offspring from mating events with the OX513A should die. The exceptions are of course if there just happen to be tetracycline at sufficiently high concentrations in a random pool of water, or a mosquito happen to be carrying a mutant version of their gene that is not functional. Both of these exceptions are extremely unlikely.

One thing I like about OX513A is that they added a fluorescent marker, so offspring from OX513A should glow red when you shine a light on them with the right wavelength. This will make it easy to figure out if any offspring survive after the initial release.
\\One last thing I want to point out is that the OX513A system functions a bit like a modern version of the sterile insect system we used to eradicate screwflies in North American back during the '60s and '70s, and would similarly require facilities to raise huge numbers of OX513A mosquitoes to be effective. In addition, OX513A rely heavily on the expression system for tTA to function as intended. Therefore, any random mutation event that renders their tTA expression system non-functional will have a huge evolutionary advantage. Thus, from an efficacy point of view, I see the OX513A as a great short-term solution (5-10 years range). Of course, the mutant mosquitoes I am talking about will be pretty similar to wild strains, so **from a safety point of view I see very little that can go wrong.**

Cheers, let me know if you have other questions or want me to clarify anything.

John Min
Ph.D. Candidate
Biological and Biomedical Sciences
Harvard Medical School

References

1. Harris, Angela F., et al. "Field performance of engineered male mosquitoes." Nature biotechnology 29.11 (2011): 1034-1037. Retrieved from: http://www.oxitec.com/wpcms/wp-content/uploads/Harris-et-al-NBT-no-page-numbers2.pdf
2. Carvalho, Danilo O., et al. "Suppression of a field population of Aedes aegypti in Brazil by sustained release of transgenic male mosquitoes." PLoS Negl Trop Dis 9.7 (2015): e0003864. Retrieved from: http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003864
3. Homem, Vera, and Lúcia Santos. "Degradation and removal methods of antibiotics from aqueous matrices–a review." Journal of environmental management 92.10 (2011): 2304-2347. Retrieved from: http://www.sciencedirect.com/science/article/pii/S0301479711001782
4. Murphy, B., et al. "Risk analysis on the Australian release of Aedes aegypti (L.)(Diptera: Culicidae) containing Wolbachia." CSIRO Report (2010). Retrieved from [PDF link]: http://www.eliminatedengue.com/library/publication/document/csiro_report_australia_2010.pdf
5. Moll, R. M., et al. "Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis."Journal of medical entomology 38.1 (2001): 29-32. Retrieved from: http://jme.oxfordjournals.org/content/38/1/29
6. Tripet, F.et al. “Competitive Reduction by Satyrization? Evidence for Interspecific Mating in Nature and Asymmetric Reproductive Competition between Invasive Mosquito Vectors.” The American Journal of Tropical Medicine and Hygiene 85.2 (2011): 265–270. PMC. Web. 12 Apr. 2016. Retrieved from: http://www.ajtmh.org/content/85/2/265.long
7. Nazni, W. A., et al. "Cross-mating between Malaysian strains of Aedes aegypti and Aedes albopictus in the laboratory." Southeast Asian Journal of Tropical Medicine and Public Health 40.1 (2009): 40.
8. Neafsey, Daniel E. et al. “‘Highly Evolvable Malaria Vectors: The Genomes of 16 Anopheles Mosquitoes.’” Science (New York, N.Y.) 347.6217 (2015): 1258522. PMC. Web. 12 Apr. 2016. Retrieved from: http://science.sciencemag.org/content/347/6217/1258522
9. “Questions & Answers on GM Mosquitoes”.Florida Keys Mosquito Control District (FKMCD) Website. (2016). Retrieved from: http://keysmosquito.org/question-answers-on-gm-mosquitoes/
10. Curtis, Zoe, et al. "Assessment of the Impact of Potential Tetracycline Exposure on the Phenotype of Aedes aegypti OX513A: Implications for Field Use." *PLoS Negl Trop Dis* 9.8 (2015): e0003999. Retrieved from: http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003999
11. Kumar, Kuldip, et al. "Antibiotic use in agriculture and its impact on the terrestrial environment." *Advances in agronomy* 87 (2005): 1-54.
12. Arensburger, Peter, et al. "The mosquito Aedes aegypti has a large genome size and high transposable element load but contains a low proportion of transposon-specific piRNAs." *BMC genomics* 12.1 (2011): 1.
13. Sethuraman, Nagaraja, et al. "Post-integration stability of piggyBac in Aedes aegypti." *Insect biochemistry and molecular biology* 37.9 (2007): 941-951.

14. Gratz, N. G. "Critical review of the vector status of Aedes albopictus."*Medical and veterinary entomology* 18.3 (2004): 215-227.
15. “Dengue in Puerto Rico”. Centers for Disease Control and Prevention. (April 22 2013). Retrieved from: http://www.cdc.gov/dengue/about/inPuerto.html
16. Phuc, Hoang K., et al. "Late-acting dominant lethal genetic systems and mosquito control." *BMC biology* 5.1 (2007): 11. Retrived from: http://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-5-11
17. Halasa, Yara A., Donald S. Shepard, and Wu Zeng. "Economic cost of dengue in Puerto Rico." *The American journal of tropical medicine and hygiene* 86.5 (2012): 745-752. Retrieved from: http://www.ajtmh.org/content/86/5/745
18. Stanaway, Jeffrey D., et al. "The global burden of dengue: an analysis from the Global Burden of Disease Study 2013." *The Lancet Infectious Diseases* (2016). Retrieved from: http://www.thelancet.com/journals/laninf/article/PIIS1473-3099(16)00026-8/abstract
19. Tomashek, Kay M., et al. "Dengue deaths in Puerto Rico: lessons learned from the 2007 epidemic." *PLoS Negl Trop Dis* 6.4 (2012): e1614. Retrieved from: http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001614
20. “Zika virus disease in the United States, 2015–2016.”Centers for Disease Control and Prevention. (April 27, 2016). Retrieved from: http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001614
21. Chan, M. "WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome”. WHO Media Centre. Retrieved from: http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/
22. Malone, Robert W., et al. "Zika virus: medical countermeasure development challenges." *PLoS Negl Trop Dis* 10.3 (2016): e0004530. Retrieved from: http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004530
23. Butler, Declan. "First Zika-linked birth defects detected in Colombia." *Nature* 531.7593 (2016): 153. Retrieved from: http://www.nature.com/news/first-zika-linked-birth-defects-detected-in-colombia-1.19502
24. Lees, Rosemary Susan et al. “Back to the Future: The Sterile Insect Technique against Mosquito Disease Vectors.” *Current Opinion in Insect Science* 10 (2015): 156–162. Retrieved from: http://www.sciencedirect.com/science/article/pii/S2214574515000905
25. World Health Organization. “WHO Virtual Press Conference on the Zika Virus.” 2016. Retrieved from: http://www.who.int/mediacentre/multimedia/zika-update-22MAR2016.pdf
26. World Travel & Tourism Council. The Economic Impact of Travel & Tourism 2015: Puerto Rico. Retrieved from: https://www.wttc.org/-/media/files/reports/economic%20impact%20research/countries%202015/puertorico2015.pdf
27. Hoffmann, A. A. et al. “Successful Establishment of Wolbachia in Aedes Populations to Suppress Dengue Transmission.” *Nature* 476.7361 (2011): 454–457. Retrieved from: http://www.nature.com/nature/journal/v476/n7361/full/nature10356.html
28. van den Hurk, Andrew F. et al. “Impact of Wolbachia on Infection with Chikungunya and Yellow Fever Viruses in the Mosquito Vector Aedes Aegypti.” *PLoS Neglected Tropical Diseases* 6.11 (2012): e1892. Retrieved from: http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001892

29. Loreto, Elgion Lucio Silva, and Gabriel Luz Wallau. “Risks of Wolbachia Mosquito Control.” Science 351.6279 (2016): 1273. Retrieved from: http://science.sciencemag.org/content/351/6279/1273.2.abstract
30. World Health Organization. “Fact Sheet: Yellow Fever.” WHO mediacentre. World Health Organization, 22 Mar. 2016. Retrieved from: http://www.who.int/mediacentre/factsheets/fs100/en/
31. Informe Semanal de Vigilancia de Chikungunya. Departamento de Salud de Puerto Rico, 2016. Retrieved from: http://www.salud.gov.pr/Estadisticas-Registros-y-Publicaciones/Estadisticas%20Chikungunya/Reporte%20ChikV%20Semana%201-2016.pdf
32. Centers for Disease Control and Prevention. “Zika Virus in Puerto Rico.” Retrieved from: http://wwwnc.cdc.gov/travel/notices/alert/zika-virus-puerto-rico
33. World Health Organization. “Chikungunya in India.” World Health Organization, 17 Oct. 2006. Retrieved from: http://www.who.int/csr/don/2006_10_17/en/
34. Margolis, Mac. “The Economic Cost of Zika Virus.” Bloomberg View. 5 Feb. 2016. Retrieved from: http://www.bloomberg.com/view/articles/2016-02-05/the-economic-cost-of-zika-virus
35. Krishnamoorthy, K. et al. “Burden of Chikungunya in India: Estimates of Disability Adjusted Life Years (DALY) Lost in 2006 Epidemic.” *Journal of Vector Borne Diseases* 46.1 (2009): 26–35. Retrieved from: http://www.mrcindia.org/journal/issues/461026.pdf
36. Oliva, Clelia F. et al. “The Sterile Insect Technique for Controlling Populations of Aedes Albopictus (Diptera: Culicidae) on Reunion Island: Mating Vigour of Sterilized Males.” *PLoS ONE* 7.11 (2012): e49414. Retrieved from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0049414
37. Lee, H. L. et al. “Mating Compatibility and Competitiveness of Transgenic and Wild Type Aedes Aegypti (L.) under Contained Semi-Field Conditions.” *Transgenic Research* 22.1 (2013): 47–57. Retrieved from: http://link.springer.com/article/10.1007%2Fs11248-012-9625-z