RESPONSE TO COMMENTS: INTROGRESSION

EPA-HQ-OPP-2019-0274

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

Several commenters stated that OX5034 genes will enter the local wild Aedes aegypti mosquito population and form a hybrid mosquito population. These commenters raised concerns about possible risks associated with these hybrid Aedes aegypti mosquitoes.

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

Three commenters (Center for Food Safety 0344, GeneWatch UK 0335, C. Cheromiah 0266) pointed out the likely genetic background of OX5034. For example, the Center for Food Safety stated that:

> "Oxitec's GE mosquitoes have been developed from a non-native strain (the Rockefeller laboratory strain[1], originally from Cuba[2]). In the Cayman Islands, this was backcrossed into a Mexico-derived genetic background[3] and it appears that this same strain was then used in Brazil and probably also in Panama. As described in Oxitec's draft Environmental Assessment for OX513A, originally submitted to the FDA, (pages 21 and 22), the GE strain OX513A was produced in 2002 by microinjection into individual embryos of *Aedes aegypti* from a Rockefeller strain background[4]. The strain was made homozygous by repeated back-crossing and then the insert was introgressed into an *Ae.aegypti* Latin strain background from Instituto Nacional de Salud Publica (INSP), Mexico. The Rockefeller strain is a common laboratory strain of *Aedes aegypti*, which appears to have been derived from a strain established in Havana, Cuba, by Carlos J. Finlay in 1881, used in the original experiments which established that *Aedes aegypti* mosquitoes are a vector for Yellow Fever[5],[6]. When Oxitec's GE mosquitoes breed with wild mosquitoes some of their other genetic characteristics will be passed on to the local wild mosquito population." (Center for Food Safety 0344 p. 11)

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[1] Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ et al. (2007) Late-acting dominant lethal genetic systems and mosquito control. *BMC Biology*, **5**: 11. doi:10.1186/1741-7007-5-1. http://www.biomedcentral.com/1741-7007/5/11

[2] Kuno, G. (2010). Early History of Laboratory Breeding of Aedes aegypti (Diptera: Culicidae) Focusing on the Origins and Use of Selected Strains. *Journal of Medical Entomology*, **47**(6), 957–971.

[3] Harris AF et al. (2011) Field performance of engineered male mosquitoes. *Nat. Biotech.*, 29(11), 1034-1037.

[4] Oxitec (2016) Draft Environmental Assessment for Investigational Use of *Aedes aegypti* OX513A. https://www.fda.gov/downloads/animalveterinary/developmentapprovalprocess/geneticengineering/geneticallyengineeredanimals/ucm487377.pdf

[5] Kuno G (2010) Early History of Laboratory Breeding of *Aedes aegypti* (Diptera: Culicidae) Focusing on the Origins and Use of Selected Strains. *Journal of Medical Entomology*, **47**(6), 957-971.

[6] Yellow Fever and the Reed Commission 1898-1901. University of Virginia Claude Moore Health Sciences Library. Historical Collection. http://www.hsl.virginia.edu/historical/medical_history/yellow_fever/index.cfm

Three commenters (GeneWatch UK 0335, Center for Food Safety 0344, Anonymous 0224,) noted that the Oxitec's stated intent was that Rockefeller/Latin genes from OX5034 mosquito would enter the local wild mosquito population.

Anonymous (0224) explained that:

> "The idea is that mass releases of GM males will mate with wild females and their offspring will contain the femalekilling trait. This genetically engineered trait is intended to make most of the female offspring of these matings die before adulthood; however the male offspring are intended to survive and breed for multiple generations. In addition, wild female pests that have mated with the released GM males will lay eggs that inherit the GM female-killing trait . . . ." (Anonymous 0224, p. 13)

GeneWatch UK (0335) noted that:

> ". . . , because the OX5034 strain is female-killing only, GE males are expected to survive for multiple generations and this will considerably increase the spread of genes from the introduced strain into the wild population. In an online presentation, Oxitec presents this as a benefit because it argues that the released laboratory-derived strain will spread insecticide susceptibility genes into the wild mosquito population[7]: however, there is no guarantee that only beneficial and no harmful traits will be spread in this way." (GeneWatch UK 0335 p. 1)

GeneWatch UK (0335) stated that:

> " . . , Oxitec has demonstrated the effects of rapid introgression of insecticide-susceptible traits in its own research and modelling of its GE agricultural pests[8,9]." (GeneWatch UK 0335 p. 9)

Other commenters (GeneWatch UK 0335, Center for Food Safety 0344) posited that data developed on the GE mosquito product, OX513A, that had been released into the environment during field testing, support the conclusion that OX5034 genes would introgress into the local wild Aedes aegypti mosquito population to form a hybrid Aedes aegypti mosquito population. For example, one commenter (GeneWatch UK 0335 p. 1) noted that with OX513A:

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[7] Martin-Rendon, E (2019) Introduction to Oxitec 2nd Generation Mosquitoes Technology Summary - Roll Back Malaria Vector Control Working Group – Geneva, 30th Jan - 1st Feb, 2019. https://endmalaria.org/sites/default/files/Enca%20Martin-Rendon.pdf

[8] Harvey-Samuel, T., Morrison, N. I., Walker, A. S., Marubbi, T., Yao, J., Collins, H. L., ... Alphey, L. (2015). Pest control and resistance management through release of insects carrying a male-selecting transgene. *BMC Biology*, **13**, 49. http://www.biomedcentral.com/1741-7007/13/49

[9] Alphey N, Bonsall MB, Alphey L (2009). Combining pest control and resistance management: synergy of engineered insects with Bt crops. *Journal of Economic Entomology*, **102**(2), 717–732.

> "The released GE males mate and produce offspring which inherit the genetically engineered late-lethality trait. This means that most (but not all) of the GE mosquitoes' offspring die at the late larval stage, in the water where the female mosquitoes lay their eggs. GeneWatch UK has repeatedly warned . . . that this partial survival rate, even if low (a reported 3 to 4% in laboratory conditions), would lead to the establishment of hybrid mosquitoes in the environment, which might possess altered properties, including the potential for enhanced disease transmission or resistance to insecticides. A recent paper, reporting monitoring of wild mosquito populations following some of Oxitec's experiments in Brazil, has confirmed that such hybrid mosquitoes have indeed spread into the area surrounding the release sites[10]." (GeneWatch UK 0335 p. 1)

The Center for Food Safety (0344) stated that:

> "A recent paper, reporting monitoring of wild mosquito populations following some of Oxitec's experiments in Brazil, has confirmed that such hybrid mosquitoes have indeed spread into the area surrounding the release sites[NOTEREF _Ref26803238 \h * MERGEFORMAT]. Because the OX5034 strain is female-killing only, GE males are expected to survive for multiple generations and this will considerably increase the spread of genes from the introduced strain into the wild population." (Center for Food Safety 0344 p. 11)

GeneWatch UK (0335) echoed this comment stating that:

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

L.M. Castro (0332) stated that:

> "A risk-benefit analysis of the proposed release of genetically modified mosquitos suggests that a significant risk of genetic material transfer exists in connection to this Experimental Permit to Combat Mosquitoes. This assertion is supported by the work of researchers from Yale University and Brazil (Evans et al.[NOTEREF _Ref26803238 \h * MERGEFORMAT]). They report that "genetic sampling from the target population six, 12, and 27–30 months after releases commenced provides clear evidence that portions of the transgenic strain genome have been incorporated into the target population" and "release of the OX513A has led to significant transfer of its genome (introgression) into the natural Jacobina population of *Aedes aegypti*. The degree of introgression is not trivial. Depending on sample and criterion used to define unambiguous introgression,

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

> from about 10% to 60% of all individuals have some OX513A genome (Tables 1 and E1). Even if the strain of Oxitec mosquitoes released in the USA is not the same as the one released in Brazil, the point is that the failings of the proposed experiment will only be known after-the-fact and the impacts will most likely be irreversible." (L.M. Castro 0332 p. 1) [Footnote referencing Evan's *et al* paper inserted]

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

> "According to a peer reviewed Yale study[NOTEREF _Ref26803238 \h * MERGEFORMAT], the GM mosquitoes released in Brazil reproduced and their GM genes contaminated the wild mosquito population. The Yale study also found that the release in Brazil did not result in a sustainable decrease in the mosquito population." (Anonymous 0329 p. 1) [Footnote referencing Evan's et al paper inserted]

GMO Free USA (0326), requesting an extension of the comment period, echoed the importance of the recent paper by Evans et al[NOTEREF _Ref26803238 \h * MERGEFORMAT] stating that:

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

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

Several commenters argued that introgression of Rockefeller/Latin OX5034 genetic material into the local wild Aedes aegypti mosquito population could present problems that should be taken into consideration. Concerns identified with the introgression of OX5034 genes into the local wild Aedes aegypti population include: the hybrid population might have greater vector competency than the original local wild Aedes aegypti population, and the hybrid population might display hybrid vigor.

The Center for Food Safety (0344) stated that while Oxitec presents the spread of OX5034 genes into the local wild Aedes aegypti mosquito population as a benefit because ". . . the released laboratory-derived strain will spread insecticide susceptibility genes into the wild mosquito population[NOTEREF _Ref26803807 \h * MERGEFORMAT]":

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

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

Several commenters (Center for Food Safety 0344, GeneWatch UK 0335, Anonymous 0226, Anonymous 0199) suggested EPA should evaluate the ramifications potentially associated with introgression of genes of the mosquito strain used to create OX5034 into the local wild Aedes aegypti mosquito population.

Anonymous (0226) noted that:

> "To create its GM mosquitoes, Oxitec started with a strain of Aedes aegypti mosquito that is commonly kept in laboratories, which probably originally came from Cuba. Before releasing the GM mosquitoes into the environment, it crossed them with wild strains from Mexico (for releases in the Americas) or Asia (for releases in Malaysia). When Oxitec's GM mosquitoes breed with wild mosquitoes some of their other genetic characteristics will be passed on to the local wild mosquito population." (Anonymous 0226 p. 11)

i. Comments raising concerns about hybrid vigor and disease transmission

The Center for Food Safety (0344) argued that:

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

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

> "The study shows that there was a gene exchange, and that in this exchange the wild mosquitoes incorporated genes from another [transgenic] variety, resulting in hybrid insects, which usually have greater vigour and are more potent . . . . What we have now is a 'super mosquito' that can grow in environments where others might not grow[12]." (L.M. Castro 0332 p. 1-2) [L.M. Castro (0332) noted that this was translated from the original Portuguese[13]]

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[11]Ekechukwu, N. E., Baeshen, R., Traorè, S. F., Coulibaly, M., Diabate, A., Catteruccia, F., & Tripet, F. (2015). Heterosis Increases Fertility, Fecundity, and Survival of Laboratory-Produced F1 Hybrid Males of the Malaria Mosquito Anopheles coluzzii. *G3* (Bethesda, Md.), **5**(12), 2693–2709. https://doi.org/10.1534/g3.115.021436

[12] https://gmwatch.org/en/news/latest-news/19144-guinea-pig-population

[13] "Troca de genes. "O estudo mostra que houve uma troca de genes, e que nessa troca os mosquitos comuns incorporaram genes de uma outra variedade, transgênica, resultando em insetos híbridos, que geralmente têm

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

> "Different strains of the same species are found in different places and some strains are more resistant to insecticides than others or better transmitters of disease (the four serotypes of the dengue virus and/or other viruses, such as chikungunya, zika and Yellow Fever). *Aedes aegypti* may transmit zika, chikungunya, yellow fever and four different serotypes of dengue, yet strains may vary significantly in their ability to transmit these tropical diseases[14],[15],[16],[17],[18],[19],[20],[21]. In the case of zika, little is known about vector strain variation and its consequences. The possible introduction of such traits needs to be considered very seriously. Harm to people's health can be increased if some serotypes or viruses can be transmitted more easily by the introduced strain than they were by the wild species already in the area, or if the strain is resistant to insecticides." (Center for Food Safety 0344 p. 11)

Anonymous (0199) stated that:

> "The demonstration of introgression resulting in increased genetic variation in Jacobina aegypti is a monumental setback. Oxitecs response is classic typology that introduced Gene's have no consequences and they assert that there is no danger because it is one

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maior vigor, são mais potentes, sobre os quais ainda não há estudos. Muito menos quanto à sua eficiência na transmissão de vírus, que pode inclusive ser maior. O que temos agora é um 'super mosquito', mais resistente, que pode se desenvolver em ambientes em que outros talvez não se desenvolveriam", avalia o biólogo José Maria Gusman Ferraz, pesquisador do Laboratório de Engenharia Ecológica da Unicamp e professor da pós-graduação do Centro Universitário da Fundação Hermínio Ometto (UniAraras)."
https://www.redebrasilatual.com.br/destaques/2019/09/irresponsabilidade-da-ctnbio-produz-super-mosquito-da-dengue/

[14] Bonizzoni M, Dunn WA, Campbell L,Olson KE, Marinotti O, James AA (2012) Strain Variation in the Transcriptome of the Dengue Fever Vector, *Aedes aegypti*. *G3*, **2**(1),103-114. http://www.g3journal.org/content/2/1/103.full

[15] Van Den Hurk AF et al. (2011) Vector Competence of Australian Mosquitoes for Yellow Fever Virus. *The American Journal of Tropical Medicine and Hygiene*, **85**(3), 446–451.

[16] Aitken TH, Downs WG, Shope RE (1977) *Aedes aegypti* strain fitness for yellow fever virus transmission. *The American Journal of Tropical Medicine and Hygiene*, **26**(5 Pt 1), 985–989.

[17] Tabachnick WJ et al. (1985) Oral Infection of *Aedes Aegypti* with Yellow Fever Virus: Geographic Variation and Genetic Considerations. *The American Journal of Tropical Medicine and Hygiene*, **34**(6), 1219–1224.

[18] Lima RS Jr, Scarpassa VM (2009) Evidence of two lineages of the dengue vector *Aedes aegypti* in the Brazilian Amazon, based on mitochondrial DNA ND4 gene sequences. *Genetics and Molecular Biology*, **32**(2), 414–422.

[19] Scarpassa VM, Cardoza TB, Cardoso RP (2008) Population Genetics and Phylogeography of *Aedes Aegypti* (Diptera: Culicidae) from Brazil. *The American Journal of Tropical Medicine and Hygiene*, **78**(6), 895–903.

[20] Vega-Rúa A, Zouache K, Girod R, Failloux A-B, Lourenço-de-Oliveira R (2014) High Level of Vector Competence of *Aedes aegypti* and *Aedes albopictus* from Ten American Countries as a Crucial Factor in the Spread of Chikungunya Virus. *Journal of Virology*, **88**(11), 6294–6306.

[21] Gonçalves CM, Melo FF, Bezerra JM, Chaves BA, Silva BM, Silva LD, … Pimenta PF (2014) Distinct variation in vector competence among nine field populations of Aedes aegypti from a Brazilian dengue-endemic risk city. *Parasites & Vectors*, **7**, 320. http://doi.org/10.1186/1756-3305-7-320

> species. A huge literature of nearly 50 years has validated significant population genetic differentiation between aegypti populations around the world and documented population variation in vector capacity for dengue, Zika and yellow fever viruses, mosquito behavior and insecticide resistance. To assert species specific variation is all the same is naive at best." (Anonymous 0199 p. 1)

J. Butler (0135) stated that:

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

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

> "For comparison, in the UK, Oxitec has been prevented from releasing a GE diamondback moth (an agricultural pest) because of concerns about the use of a North American background strain, which is subject to controls under plant pest control regulations."[22] (GeneWatch UK 0335 p. 10, Center for Food Safety 0344 p. 12)

2. Comments Rebutting Concerns about Introgression Resulting in Hybrid Vigor and Disease Transmission

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

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

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

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

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[22] HSE (2011) Letter to Oxitec. 5 December 2011. Obtained by GeneWatch UK as the result of a Freedom of Information request.

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

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

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

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) responding to the Evans et al[NOTEREF _Ref26803238 \h * MERGEFORMAT] statement that "introgression may introduce other relevant genes such as for pesticide resistance" stated that:

> " . . . OX513A is susceptible to standard insecticides (for example, pyrethroids and organophosphates) used for mosquito control. . . . (Carvalho et al., 2015)[23] . . . . (Patil et al., 2018)[24]." (N. Rose 0341 p. 6)

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

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

N. Rose, Head of Regulatory Science, Oxitec, Ltd., (0341) responding to the Evans et al[NOTEREF _Ref26803238 \h * MERGEFORMAT] statement that it is not known "what impacts introgression from a transgenic strain of Ae. aegypti has on traits of importance to disease control and transmission" stated that there is an:

> " . . . extensive body of literature that demonstrates that many of the factors most likely to affect vector competence are not genetic, but environmental, relating to the mosquito's microbiome and immune response, and relating to the genetics of the virus

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[23] Carvalho DO., McKemey AR., Garziera L., Lacroix R., Donnelly CA., Alphey L., Malavasi A., Capurro ML. 2015. Suppression of a Field Population of Aedes aegypti in Brazil by Sustained Release of Transgenic Male Mosquitoes. *PLoS Negl Trop Dis* 9:e0003864. DOI: 10:1371/journal.pntd.0003864.

[24] Patil PB., Gorman KJ., Dasgupta SK., Reddy KVS., Barwale SR., Zehr UB. 2018. Self-Limiting OX513A Aedes aegypti Demonstrate Full Susceptibility to Currently Used Insecticidal Chemistries as Compared to Indian Wild-Type Aedes aegypti. *Psyche (New York)* 2018. DOI: 10.1155/2018/7814643.

rather than the vector (Tabachnick, 2013[25]; Palmer, Varghese & Van Rij, 2018[26]; Souza-Neto, Powell & Bonizzoni, 2019[27])." (N. Rose 0341 p. 5)

[25] Tabachnick WJ. 2013. Nature, nurture and evolution of intra-species variation in mosquito arbovirus transmission competence. *International Journal of Environmental Research and Public Health* 10:249–277. DOI: 10.3390/ijerph10010249.

[26] Palmer WH., Varghese FS., Van Rij RP. 2018. Natural variation in resistance to virus infection in dipteran insects. *Viruses* 10:118. DOI: 10.3390/v10030118.

[27] Souza-Neto JA., Powell JR., Bonizzoni M. 2019. Aedes aegypti vector competence studies: A review. *Infection, Genetics and Evolution* 67:191–209. DOI: 10.1016/j.meegid.2018.11.009.