Overall it appears that an adequate biosafety risk examination of this particular strain of *Wolbachia* (wAlb) in this particular mosquito (*Aedes aegypti*) has not been performed by the applicants. As '*Wolbachia causes very different responses depending on the host*' then each time *Wolbachia* is inserted into a new host it should be fully assessed. The information in publications from numerous independent scientists that cite various risks regarding the use of *Wolbachia* as a vector (i.e., mosquito) control method should be cause for concern.

Below is specific feedback to comments in the email received, as well as the EPA response to public comments from August 2016 that were cited in response to third-party scientist publications that express concerns or note potential issues with *Wolbachia*, including several specifically citing risks for its use in vector control.

1. **Feedback to comments in email received:**

   Email comment – *"Citation 5: This research is for a completely different organism, Spodoptera exempta (African armyworm), not mosquitoes, and Wolbachia causes very different responses depending on the host. This information, while interesting, cannot be used to make predictions about mosquitoes."*

   Feedback – These two publications listed here are: (1) specific to mosquitoes and (2) involve the wAlbB strain of *Wolbachia*. These are not being cited to make predictions, however they involve mosquitoes as well as the wAlbB strain, and are relevant to a proper risk assessment:

   - *Wolbachia* Strain wAlbB Enhances Infection by the Rodent Malaria Parasite Plasmodium berghei in Anopheles gambiae Mosquitoes https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/
   - *Wolbachia* Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965

   As noted in EPA comments '*Wolbachia causes very different responses depending on the host*', therefore, every insertion of *Wolbachia* into a new host – like *Aedes aegypti* - should be tested and fully assessed given the interactions are unknown and unpredictable.

Email comment - *Citation 12: With respect to the Wolbachia phage encoding a toxin from the black widow spider, this comment is referring to Citation 12 ("Eukaryotic association module in phage WO genomes from Wolbachia"). In this study, the WO-B phage in wAlbB strain mosquitoes were not studied. Only moth and parasitoid wasp WO phage were researched. Aedes aegypti wAlbB strain does have a WO-B phage associated with it; however, phage are specific to their hosts, horizontal gene transfer is happening on evolutionary time scales, there is no indication that the widow spider toxin sequence is expressed in the Wolbachia infection, and it is not reported that the WO-B phage in wAlbB Aedes aegypti produce the toxin from black widow spider. Also, most importantly, viruses*

have been shown to incorporate host sequences numerous times, but this is the first report of a virus of an obligate intercellular parasitic bacterium having sequences from both hosts: bacterial and eukaryotic. The widow spider toxin is a huge multimeric toxin with the entire 150kD monomer needing to be expressed and binding to form a tetramer to have full toxin activity. The sequence detected in the prophage sequence is only the C-terminus (maybe 18 kD) of the entire monomeric protein (150 kD). This C-terminus has been implicated in passage through membranes to release the toxins when produced in the spider. Furthermore, there is no evidence that Wolbachia alone are being transferred to animals when a female mosquito bites and takes a blood meal from an animal.

Feedback - Granting an experimental use permit notwithstanding the current lack of understanding of potential adverse effects that may result from the transfer of potentially harmful genes would seem to be inconsistent with EPA's regulatory obligations under FIFRA. It is well known that phage have ability to insert its genes into bacterial genomes. The WO phage (once inserted it's called a prophage) can and does insert into the *Wolbachia* genome – this has been established as per this 2016 publication:

- Eukaryotic association module in phage WO genomes from Wolbachia http://www.nature.com/articles/ncomms13155

This results in genetic transformation that is unknown and uncharacterized, i.e., an unknown transgenic genome. The argument that the spider toxin will not be expressed because it is not the full genome is not the point. What is the C-terminus encoding for? Why is it there? What is the impact of it being there? It is imperative that these questions be addressed and investigated prior to *Wolbachia* being released into the environment in human biting mosquitoes.

Email comment – *Citation 13, 14, and 15: The Wolbachia pipientis strains associated with River blindness and lymphatic filariasis are in different clades than the wAlbB, and the wAlbB strain is not associated with these diseases.*

Feedback – The proponents of *Wolbachia* as a potential vector control method continuously cite that ~60% of insects may be infected with *Wolbachia* and therefore it is safe.

This estimate, which is at the highest end of the range of 20% to 60% listed in various publications, covers all *Wolbachia* strains including the clades of *Wolbachia* that are associated with river blindness that has infected over 30 million people in a single year and with lymphatic filariasis that infects an estimated 120 million people in tropical and subtropical areas according to the World Health Organization.

The mechanisms of action of any of the clades of *Wolbachia* are not completely understood and given these statistics and publications, extreme caution should be taken with the artificial introduction into a human biting mosquito.

These publications discuss Wolbachia's association with these devastating diseases that impact tens of millions of people worldwide:

- Onchocerciasis: the Role of Wolbachia Bacterial Endosymbionts in Parasite Biology, Disease Pathogenesis, and Treatment https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3131055/
- The Role of Endosymbiotic Wolbachia Bacteria in the Pathogenesis of River Blindness http://www.nature.com/news/2002/020304/full/news020304-9.html
- Short Course, High Dose Rifampicin Achieves Wolbachia Depletion Predictive of Curative Outcomes in Preclinical Models of Lymphatic Filariasis and Onchocerciasis http://archive.lstmed.ac.uk/6918/
- Wolbachia bacteria in filarial immunity and disease. https://www.ncbi.nlm.nih.gov/pubmed/11472559

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2. **Feedback to Comments in EPA's Response to Comments Received on the April 26, 2016, Notice of Receipt for an Amendment and Extension to Experimental Use Permit 88877-EUP-2 (Docket ID Number: EPA-HQ-OPP-2015-0374; FRL-9944-96) cited in the email.** EPA's response to comments Public Comment #3.

EPA Response to Public Comment #2 – *"The Wolbachia-based Aedes aegypti product proposed for experimental field trials mimics the cytoplasmic incompatibility phenotype as known from numerous insects and other arthropods; while a single report exists in the literature indicating natural infection of Aedes aegypti, it is estimated that greater than 1 million species extant in the environment harbor naturally occurring Wolbachia strains. This presence and degree of exposure to a variety of organisms without documented negative impacts suggests that the product under consideration by EPA is also likely to pose minimal probability of adverse effects to humans and the environment."*

Feedback - This seems to state the following:

1. There are "naturally occurring" *Wolbachia* in over 1 million species "without documented negative impacts," therefore, *Wolbachia* in Aedes aegypti is "likely to pose" minimal risk; and
2. *Wolbachia* strains associated with River Blindness and lympatic filariasis have been well documented, yet they are different to the *Wolbachia* strain being utilized, so there should be no risk.

These points are logically inconsistent and are mutually incompatible. On the one hand, this comment says there are no risks because of no documented negative effects. Yet there are obviously negative documented negative effects, yet these don't count.

These publications discuss *Wolbachia's* association with devastating diseases that impact tens of millions of people worldwide, as well as its potential role in driving pathogen increase in its hosts:

- Onchocerciasis: the Role of Wolbachia Bacterial Endosymbionts in Parasite Biology, Disease Pathogenesis, and Treatment
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3131055/
- The Role of Endosymbiotic Wolbachia Bacteria in the Pathogenesis of River Blindness
  http://www.nature.com/news/2002/020304/full/news020304-9.html
- Wolbachia bacteria in filarial immunity and disease.
  https://www.ncbi.nlm.nih.gov/pubmed/11472559
- Short Course, High Dose Rifampicin Achieves Wolbachia Depletion Predictive of Curative Outcomes in Preclinical Models of Lymphatic Filariasis and Onchocerciasis
  http://archive.lstmed.ac.uk/6918/
- Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?
  http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004182
- Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis
  http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965
- Wolbachia increases susceptibility to Plasmodium infection in a natural system
  http://rspb.royalsocietypublishing.org/content/281/1779/20132837
- Wolbachia Strain wAlbB Enhances Infection by the Rodent Malaria Parasite Plasmodium berghei in Anopheles gambiae Mosquitoes
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/
- Wolbachia in a major African crop pest increases susceptibility to viral disease rather than protects.
  https://www.ncbi.nlm.nih.gov/pubmed/22731846

EPA Response to Public Comment #2 – *"...it is estimated that greater than 1 million species extant in the environment harbor naturally occurring Wolbachia strains. This presence and degree of exposure to a variety of organisms without documented negative impacts suggests that the product under consideration by EPA is also likely to pose minimal probability of adverse effects to humans and the environment."*

Feedback – All *Wolbachia* are not created equal, yet those that wish to see it used as a vector control method consistently note that up to ~60% of insects (some estimates of *Wolbachia* penetration are significantly lower) may be infected with it.

The fact they are not equal is supported by the fact that teams interested in *Wolbachia* as a vector control method tested several different *Wolbachia* strains in *Aedes* to get to one that

gave the desired lethality in offspring. Some strains gave no lethality at all, and other strains resulted in less than desired lethality.

More importantly, when *Wolbachia*-infected filarial worms invade the human body through the bites of insects, namely mosquitoes and flies, human immune responses occur which lead to river blindness and lymphatic filariasis that impact the lives of tens of millions of people across the globe. Here are some of the publications that cover *Wolbachia* and these diseases:

- Onchocerciasis: the Role of Wolbachia Bacterial Endosymbionts in Parasite Biology, Disease Pathogenesis, and Treatment https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3131055/
- The Role of Endosymbiotic Wolbachia Bacteria in the Pathogenesis of River Blindness http://www.nature.com/news/2002/020304/full/news020304-9.html
- Wolbachia bacteria in filarial immunity and disease. https://www.ncbi.nlm.nih.gov/pubmed/11472559
- Short Course, High Dose Rifampicin Achieves Wolbachia Depletion Predictive of Curative Outcomes in Preclinical Models of Lymphatic Filariasis and Onchocerciasis http://archive.lstmed.ac.uk/6918/

Therefore, the logic that the *"presence and degree of exposure to a variety of organisms without documented negative impacts suggests that the product under consideration by EPA is also likely to pose minimal probability of adverse effects to humans and the environment"* should be reconsidered as it is not clear that statement is accurate, especially with respect to these flies and mosquitoes that are the source for the widespread river blindness and lymphatic filariasis diseases.

In the case of wAlbB specifically, what percentage of insects have been infected with it? There are documented cases of this bacterium driving pathogen production higher in the hosts it invades including those listed below that are (1) specific to mosquitoes and (2) also involve the wAlbB strain of *Wolbachia*:

- *Wolbachia* Strain wAlbB Enhances Infection by the Rodent Malaria Parasite Plasmodium berghei in Anopheles gambiae Mosquitoes https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/
- *Wolbachia* Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965

EPA Response to Public Comment #2 – *"...while a single report exists in the literature indicating natural infection of Aedes aegypti"*

Feedback –The report that is cited in the EPA response links to a NCBI Taxonomy page (here).

There is another paper that was published in 2009 (Klasson et al) that raises concerns regarding natural infection of *Aedes aegypti*:

- Horizontal gene transfer between *Wolbachia* and the mosquito Aedes aegypti https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-10-33

Here is excerpt from this paper by Klasson et al:

- "We have discovered a case of horizontal gene transfer (HGT), involving two adjacent genes, between the genomes of *Wolbachia* and the currently *Wolbachia*-uninfected mosquito Aedes aegypti, an important human disease vector. The lower level of sequence identity between *Wolbachia* and insect, the transcription of all the genes involved, and the fact that we have identified homologs of the two genes in another Aedes species (Ae. mascarensis), suggest that these genes are being expressed after an extended evolutionary period since horizontal transfer, and therefore that the transfer has functional significance. The association of these genes with *Wolbachia* prophage regions also provides a mechanism for the transfer. The data support the argument that HGT between *Wolbachia* endosymbiotic bacteria and their hosts has produced evolutionary innovation."

Given this publication has unknown implications regarding artificially inserting *Wolbachia* back into *Aedes aegypti*, has EPA been provided test data or other evidence that substantiates or negates its conclusions?

Of note in the 2016 publication by Bordenstein cited earlier - Eukaryotic association module in phage WO genomes from Wolbachia http://www.nature.com/articles/ncomms13155 - the authors stated *'Among this subset with eukaryotic sequence homology, the protein domains are almost exclusively found in the phage eukaryotic association module (EAM). An EAM has never before been reported in bacteriophage genomes, to our knowledge, possibly because phages of obligate intracellular bacteria occupy a unique eukaryotic-enclosed niche and are relatively understudied'.*

This is an important observation, along with other evidence of HGT that indicates there is still a lot to understand about the interaction of *Wolbachia* with its host and the implications.

EPA Response to Public Comment #3 – *"The wAlbB Aedes aegypti strain is not intended to affect the competency of the vector to transmit viral agents. Currently, there is no compelling evidence that wAlbB in Aedes aegypti does affect the capacity of the vector to transmit disease agents."*

Feedback – Has the vector competency work been completed to establish this? As the EPA comment highlighted, it is known that '*Wolbachia causes very different responses*

depending on the host', so any new *Wolbachia* host interaction should be fully investigated including any change in disease vectoring capacity which these publications suggest occurs:

- Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?
  http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004182
- Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis
  http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965
- Wolbachia increases susceptibility to Plasmodium infection in a natural system
  http://rspb.royalsocietypublishing.org/content/281/1779/20132837
- Wolbachia Strain wAlbB Enhances Infection by the Rodent Malaria Parasite Plasmodium berghei in Anopheles gambiae Mosquitoes
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/
- Wolbachia in a major African crop pest increases susceptibility to viral disease rather than protects.
  https://www.ncbi.nlm.nih.gov/pubmed/22731846

EPA Response to Public Comment #3 – *"Even with this strategy of deliberate male and female releases, failure to establish Wolbachia-infected populations has occurred in some instances."*

Feedback – This reference to "failure to establish *Wolbachia*-infected populations" occurring in "some instances" is based on a different clade of *Wolbachia* known as wMel from D melanogaster, whereas MosquitoMate uses a wAlbB strain from Aedes albopictus. With this in mind, have there been tests done to show it will not happen specific to the CI approach with wAlbB *Wolbachia* in Aedes aegypti?

This is relevant given the entire strategy depends on not releasing infected females, and the consequences are failure of the approach to control the *Aedes aegypti* population and the spreading of a bacterium into the environment in a human biting mosquito with unknown potential adverse effects.

EPA Response to Public Comment #3 – *"Establishing a wAlbB Aedes aegypti population is highly unlikely because only males are released with very few potential accidental female releases, i.e., less than 1 female per 250,000 males (U.S. EPA, 2015a and 2015b)."*

Feedback – This equation results in a 99.9996% sex sorting ratio. Publications show sex sorting efficiency in mosquitoes range from 96-99.99%, with some approaches as low as 85% (http://johnwhock.com/wp-content/uploads/2012/09/instr_5412_separator.pdf).

With a proprietary approach Oxitec has achieved 99.99% efficiency at scale. What scale is this reported 99.9996% sex sorting ratio achieved? Is this based on releases of thousands or millions of mosquitoes? Are there validated data demonstrating this level of efficiency that are publicly available? We are aware of no published data showing this sorting efficiency that has been afforded independent review.

EPA Response to Public Comment #3 – *"According to Xi et al. (2005), in caged releases of wAlbB Aedes aegypti females with uninfected males, a minimum of 20% of females needed to be released to establish the Wolbachia infection after seven generations. All releases below 20% in that cage experiment resulted in failure of the wAlbB infection to be established in the population."*

Feedback: The Xi et al (2005) paper were very small cage trials (100 adults per cage) and do not reflect conditions in the field. Additionally as noted in the EPA response, the Xi et al study did not test for the unintentional release of wAlbB females right next to wAlbB males in the environment, but rather wAlbB females into a population of uninfected males in cages. Given the compatibility between infected wAlbB males and wAlbB females, a single female release could theoretically lead to the persistence of *Wolbachia* in subsequent generations of *Aedes aegypti*.

The entire strategy employed by MosquitoMate depends on not releasing females, and the risk of accidental female release has not been fully assessed. This could result in a failure of the technology to control the *Aedes aegypti* population, and lead to the spread of a bacterium that is not fully understood into the environment and into a pervasive human biting mosquito.

Furthermore, understanding what happens in naturally in the environment with respect to the spread of *Wolbachia* is worthy of consideration as opposed to small cage trial results that tested for something completely different than the risk of accidental releases of wAlbB females alongside compatible wAlbB males. Notably, it is impossible that when *Wolbachia* invades a population it does so by infecting over 20% of that population at one time. It starts with a few individuals that somehow get infected with *Wolbachia* (there is still a lot of debate as to how *Wolbachia* spreads between species) and then it spreads through the entire population over time.

EPA Response to Public Comment #3 – *"It may be possible to establish a wAlbB Aedes aegypti population with use of the wAlbB strain. This, however, would only be possible if substantial numbers of females were released into an ecosystem along with repeated male wAlbB Aedes aegypti releases. The release of females is strictly controlled in the quality*

control procedures during mechanical separation of pupae and microscopic inspection of sorted pupae (U.S. EPA, 2015a)."

Feedback – The risk of releasing females increases with the scale of deployment. What are the QC checks in place and can they be scaled reliably? We know of no published data independently validating the sorting efficiency for this strain. As the consequences of female releases are unknown (Will it spread under field conditions? What happens if it spreads? Can it affect vectorial capacity?), the sorting efficiency is of paramount importance and therefore requires utmost scrutiny and validation.

Additionally please see previous response regarding assumption that the establishment of a *w*AlbB *Aedes aegypti* population is only possible "if substantial numbers of females were released into an ecosystem". This is an assumption based on 5 small cage trials and is not consistent or relevant to how *Wolbachia* naturally invades a species.

EPA Response to Public Comment #3 – *"In addition, UKDE monitors the environment near its rearing facilities for inadvertent release or escape of female Aedes aegypti wAlbB. As with any mechanical separation technique for mosquitoes, continual monitoring and quality assurance measures are paramount for ensuring that only males are released."*

Feedback - How is this accomplished? What sorts of assay are used with what accuracy, and what are the limits of detection? What do you do if you find female *Aedes aegypti* infected with wAlbB?

EPA Response to Public Comment #3 – *"Currently, there is no compelling evidence that wAlbB in Aedes aegypti does affect the capacity of the vector to transmit disease agents. A few published manuscripts have discussed West Nile virus (WNV) and Plasmodium titers increasing in Wolbachia-positive strains, but Aedes aegypti are not a natural malaria vector and Aedes aegypti do not generally carry WNV (Hughes et al., 2014a). Because Aedes aegypti are not a natural malaria vector, research showing the effects in Culex pipiens cannot be used to assume this is true for Aedes aegypti. Additionally, Hughes et al. (2012) discusses Plasmodium infections in Anopheles gambiae with wAlbB, and this is not applicable to the situation with Aedes aegypti."*

Feedback – As highlighted in the EPA comment '*Wolbachia causes very different responses depending on the host*', therefore every insertion of *Wolbachia* into a new host, as is the case with *Aedes aegypti*, should be fully assessed and tested especially if the interactions are unknown and unpredictable. Have biosafety tests been run by the applicant to confirm wAlbB in *Aedes aegypti* does not affect the capacity of the vector to increase pathogen

production and transmit disease agents as various publications suggest?

This is an important question when the following publications that show *Wolbachia* can increase viral load in its hosts are taken into consideration:

- Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?
  http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004182
- Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis
  http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965
- Wolbachia increases susceptibility to Plasmodium infection in a natural system
  http://rspb.royalsocietypublishing.org/content/281/1779/20132837
- Wolbachia Strain wAlbB Enhances Infection by the Rodent Malaria Parasite Plasmodium berghei in Anopheles gambiae Mosquitoes
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/
- Wolbachia in a major African crop pest increases susceptibility to viral disease rather than protects.
  https://www.ncbi.nlm.nih.gov/pubmed/22731846

EPA Response to Public Comment #5 – *"The likelihood of wAlbB Aedes aegypti survival is considered low given that males are dead end hosts, the small number of potential accidental female releases, and bidirectional cytoplasmic incompatibility. As mentioned in the Response to Public Comment #3, at least 20% of the population of females would need to be released to establish a wAlbB Aedes aegypti constant population."*

Feedback – Males are dead end hosts only when sex-sorting is 100%. Notably any *Wolbachia* female mosquitoes released will be pre-mated (as adults are released that are over 2 days old), mature, ready to feed on human hosts, and lay eggs that can survive to adulthood.

With respect to the *"at least 20% of the population of females would need to be released to establish a wAlbB Aedes aegypti constant population",* as mentioned earlier the Xi et al (2005) paper were very small cage trials (100 adults per cage) and do not reflect conditions in the field. Additionally, as noted in the EPA response, the Xi et al study did not test for the unintentional release of wAlbB females right next to wAlbB males, but rather wAlbB females into a population of uninfected males in cages. Given the compatibility between infected wAlbB males and wAlbB females, a single female release could theoretically lead to the persistence of *Wolbachia* in subsequent generations of Aedes aegypti.

The entire strategy employed by MosquitoMate depends on not releasing females, and the risk of accidental female release has not been fully assessed. This could result in a complete failure of the technology to control the *Aedes aegypti* population, and lead to the spread of

a bacterium that is not fully understood into the environment via a pervasive human biting mosquito.

Furthermore, understanding what happens in naturally in the environment with respect to the spread of *Wolbachia* is worthy of consideration as opposed to small cage trial results that tested for something completely different than the risk of accidental releases of wAlbB females alongside compatible wAlbB males. Notably, it is impossible that when *Wolbachia* invades a population it does so by infecting over 20% of that population at one time. It starts with a few individuals that somehow get infected with *Wolbachia* (there is still a lot of debate as to how *Wolbachia* spreads between species) and then it spreads through the entire population over time.

EPA Response to Public Comment #7 – *"As discussed in Brelsfoard and Dobson (2009), in Drosophila melanogaster, the wMel strain of Wolbachia may influence the susceptibility of this fly to RNA-type viruses. It is further hypothesized that this phenomenon may occur in other host species harboring Wolbachia; however, no direct evidence is provided. Aedes aegypti reared by UKDE for release of Wolbachia-infected males are checked for the presence of infectious virus particles as part of the manufacturing process. Any significant changes that may occur with respect to favoring the presence of a pathogen would therefore be noted as part of the quality assurance protocols in place."*

Feedback – Checking for the presence of the virus in a facility where the females are kept in cages and have limited exposure to human viruses (such as Zika, dengue, chikungunya, yellow fever etc.) is not a test for influences on susceptibility.

Has the potential phenomenon of *Wolbachia* impacting susceptibility of RNA-type viruses to hosts such as flies been specifically tested in the *Wolbachia* wAlbB strain of *Aedes aegypti* mosquitoes?

EPA Response to Public Comment #7 – *"Calvitti et al. (2015) showed that wAlbA Aedes albopictus in dense rearing conditions did not decrease the cytoplasmic incompatibility (CI) effect. Islam and Dobson (2006) also showed that rearing Aedes albopictus with Wolbachia under crowded, low food conditions did not impact the CI effect. Yamada et al. (2007) cited by Oxitec, Ltd. refers to the effect in Drosophila, not mosquitoes."*

Feedback - *Aedes albopictus* has adapted to the presence of wAlbA/B over a long period of time, whereas *Aedes aegypti* has only recently been infected with wAlbB. Hence the example from Yamada et al (2007) is relevant because it suggests that a similar effect may occur in *Aedes aegypti* – that is that males that developed faster had almost complete cytoplasmic incompatibility (CI) whereas those that developed more slowly lost much of the

CI effect. This should be tested for and evaluated properly in *Aedes aegypti* under a range of different rearing conditions and male development time; otherwise there is a risk of releasing males with incomplete CI causing a failure to control the *Aedes aegypti* mosquito population.

In summary, examining these biosafety risks with this strain, in this species of mosquito, and with the relevant disease vectors is a completely possible thing to do. To require that an applicant have done so, prior to being approved for field trials, is not only reasonable but a necessary standard.

This should especially be required in a case, as we have here, in which so many serious scientific journal articles attest to significant risks to human health posed by *Wolbachia*, in other human biting insects and with a variety of disease causing organisms.