EPA Must Revoke the Improperly Granted *Wolbachia Aedes aegypti* Experimental Use Permit

EPA's Office of Pesticide Programs improperly granted an Experimental Use Permit (EUP) to MosquitoMate allowing releases of millions of Wolbachia-infected *Aedes aegypti* mosquitoes in California and Florida [1]. Oxitec and other commenters submitted substantive comments on the record identifying significant concerns and critical risks associated with the proposed EUP. Notwithstanding these comments, EPA granted the requested EUP to MosquitoMate.

EPA can immediately remedy this improper regulatory approval and all other current *Wolbachia* EUPs. Pursuant to FIFRA Section 5(e) (7 U.S.C. § 136c(e)), the EPA Administrator may revoke an EUP immediately if he finds that its terms or conditions are being violated or that its terms or conditions are inadequate to avoid unreasonable adverse effects on the environment. (See also 40 C.F.R. § 172.10(a)) EPA's regulations provide that a permittee may "contest" the notice of revocation by requesting "an opportunity to confer with the Administrator." The Administrator will issue a final decision within 20 days after such conference. (40 C.F.R. § 172.10(c)) Neither FIFRA nor EPA's regulations provide for a formal hearing to object to revocation of an EUP.

EPA's granting of an experimental use permit to MosquitoMate for an *Aedes aegypti* artificially infected with *Wolbachia pipientis* was premature and did not properly assess the potential adverse impacts to the environment that may result from releases of *Aedes aegypti* artificially infected with a bacterium that is not native to the *Aedes aegypti* microbiome. EPA did not conduct a necessary rigorous assessment of the potential adverse impacts to human health and the environment that could result from uncontained release of *Aedes aegypti* containing this non-native strain of bacteria.

Of particular concern is the fact that horizontal gene transfer could result in *Wolbachia* effectively introducing over one thousand new genes into these modified *Aedes aegypti*. The potential genetic modification to *Aedes aegypti* that may result from use of the wAlbB *Wolbachia* bacterium is wholly undefined. Several studies have shown that horizontal gene transfer between *Wolbachia* and their insect hosts may result in gene transfers ranging from nearly the entire *Wolbachia* genome (>1 megabase) to short insertions (<500 base pairs) into various hosts [2-5]. Effectively the *Wolbachia* IIT approach could introduce over one thousand new genes into *Aedes aegypti* with unknown consequences, and if they provide a positive selection to the mosquito in the environment this could result in novel strains pervading and spreading through the population.

Critical questions about the *Wolbachia Aedes aegypti* that EPA did not answer prior to granting the EUP include the following:

- Can the *Wolbachia* mosquito escape the confined conditions in which it is reared?
- What is the likelihood that the *Wolbachia* mosquitoes will survive, reproduce, and disperse once released into the environment? Modelling has shown that conditions of lower competition can favor the spread of *Wolbachia*-infected females [6-8]. In other words, as a mosquito population is reduced, or if a population is already low, the chances of *Wolbachia* invading the wild population are increased.

- What are the potential impacts of the *Wolbachia* mosquitoes in the environment, including on humans? The whole genome of *Wolbachia* can transfer to a host genome, meaning a host mosquito could be transformed with over one thousand new genes with unpredictable results [2-5]. Horizontal gene transfer (HGT) can transfer genes between *Wolbachia* and its host in *Aedes aegypti* [12] and several other mosquito species [13, 25]. Therefore, *Wolbachia* can genetically transform its host with functional genes with currently unknown consequences. EPA did not require any investigation or risk assessment of these potential consequences.

  Moreover, widespread recombination occurs throughout the *Wolbachia* genome [14], increasing the likelihood of genes changing as the *Wolbachia* evolves. Moreover, temperature impacts *Wolbachia*-malaria interaction in mosquitoes suggesting impact of transfection might vary across diverse environments [17]. Also, it has been shown that *Wolbachia* enhances West Nile virus infection in the mosquito *Culex tarsalis*. This introduces the possibility that the *Wolbachia* infection could spread to *Culex* populations in areas where West Nile virus is a concern [18]. Similarly, *Wolbachia* can enhance malaria parasite infection in two genera of mosquitoes [19-21].

- What are the likely consequences for the surrounding environment, should the *Wolbachia* mosquitoes survive and establish in the environment? There is evidence that male age and overcrowding during development (*i.e.*, under mass rearing conditions required to produce enough males for IIT to be effective) can reduce the cytoplasmic incompatibility effect in certain insects, rendering the males fertile [26] and able to spread the *Wolbachia* infection through surviving females. EPA should have required a study to assess whether this effect is likely to be seen in *Aedes aegypti*. EPA should have required additional analysis to assess interactions of *Aedes aegypti*, potential pathogens, and *Wolbachia*, to ensure that *Wolbachia* does not ultimately select for more dangerous pathogens in *Aedes aegypti* [22].

EPA should have, but did not, evaluate these and other critical risk assessment questions before it granted the *Wolbachia* EUP extension, which allows the release of 14 million of these inadequately evaluated *Wolbachia*-infected *Aedes aegypti* into the environment. As is demonstrated in the published literature referenced below, and as Oxitec and other commenters demonstrated on the record, releases of such massive numbers of these artificially infected *Aedes aegypti* could result in serious unreasonable adverse effects to the environment.

References

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