Top 10 Problems with Genetically Modified Mosquito OX5034

Genetically modified mosquitoes are planned for release in the very near future. There are many facts about Oxitec's OX5034 mosquitoes that the general public is not aware of. Below are some important facts that residents and tourists should be aware of.

1.OX5034 is very different from the previous genetically modified mosquito, OX513A, that was planned for release.

During a 2016 referendum residents of the Keys voted on the release of OX513A. The residents were told that these mosquitoes would all be male, which do not bite, and would not have any offspring that survived to adults. Essentially, once they were released they would all die and their offspring would all die with them and so wouldn't remain in the environment. Although it was later found that this was false, some females would be released and around 5% of the offspring would survive to adults(1), this is far different than the OX5034 where 50% of the offspring survive to adults. The general public have never voted on the release of OX5034.

2. Female OX5034 mosquitoes may be released.

Like with the OX513A, the public is being told that only male OX5034 mosquitoes will be released. The plan is to release as many as 1 billion, supposedly all male, mosquitoes. The EPA has even stated that if even 1 female is found the experiment will immediately stop. Yet, a recent study suggests females will be released.(2) Identification of females during a trial would rely on capturing females. Yet, traps often capture a low number of mosquitoes. This means that if a trial were to take place hundreds or even thousands of OX5034 females could exist in the environment before a single female is even captured and the experiment ends. Allowing this experiment to continue, therefore, would be irresponsible and a potential danger to the residents of the Florida Keys and perhaps all of Florida and beyond.

3. OX5034 mosquitoes may increase mosquito-borne diseases.

A recent study found a type of genetically modified mosquito has a greater ability to transmit malaria.(3) Evidence also suggests some Aedes aegypti are able to effectively transmit dengue(4), Zika virus(5) or chikungunya(6) and others are not. Testing to determine the ability of OX5034 to transmit mosquito-borne diseases has not been done. OX5034 mosquitoes may increase mosquito-borne diseases in a number of ways. OX5034 mosquitoes are lab-raised so their eggs are likely disease free, but then these mosquitoes go out in the wild and mate with wild females. If a wild female is infected with a disease, like Zika, and a male mates with her, he can acquire Zika from the female(7). Since a male can mate over a dozen times in his life he is able to spread a disease like Zika. Therefore, releasing a billion males able to spread diseases is incredibly risky. The wild females that mate with the OX5034 males then have male, and likely some female, offspring. If the wild female has dengue, or various other mosquito-borne diseases, she can then pass that dengue to her male offspring through transovarial transmission(8). Now male Aedes aegypti can harbor mosquito-borne diseases like dengue

fever(9) and chikungunya, for example, and if males are infected they could sexually transmit(10) these mosquito-borne diseases to other females(11). No testing has been done to determine if OX5034 have higher rates of mosquito-borne diseases via transovarial transmission, or are better able to transmit mosquito-borne diseases via venereal transmission compared to wild mosquitoes. It's not just human diseases that are a concern. The Aedes aegypti can spread avian malaria putting endangered birds such as the Southern bald eagle and Roseate tern at risk. It also spreads canine and feline heartworm putting pets at risk.

When the OX5034 males mate with the wild females they will receive sperm from the male. The receipt of seminal fluid proteins that are transferred from males to females(12) along with sperm during copulation cause changes in host-seeking and feeding behavior, blood digestion rate, increase blood meal size, re-mating behavior, stimulates egg development, increase fecundity and increase life expectancy which may impact capacity for disease transmission. If the seminal fluid of a OX5034 mosquito is altered due to the transformation process, this could potentially cause a wild female Aedes aegypti to feed more and/or on more hosts or increase life expectancy therefore causing the potential for increased disease spread. No testing has been provided for this potential risk.

4. OX5034 mosquitoes may increase allergies.

Oxitec plans to initially increase the number of Aedes aegypti in the test areas. Mosquito-derived allergens are present in air(13), and allergy to insects(14) has a significant bearing on the clinical characteristics of allergic bronchial asthma patients(15). This could increase the number or severity of Type I allergic respiratory disorders. Based on the data provided to the EPA it does not appear that testing has been done to determine whether there is an increase in quantity of inhalant allergens or new potential inhalant allergens in the OX5034 mosquitoes.

Oxitec's second generation GMO mosquitoes have synthetic DNA based on a fusion of sequences from E.coli and the Herpes Simplex virus and express a synthetic protein. They also have other synthetic DNA based on sequences from coral which express another synthetic protein. In Oxitec's data provided to the FDA(16) for their first generation GM mosquitoes, OX513A, they provided these sequences. When these sequences were put into the Structural Database of Allergenic Proteins(17), using FAO/WHO Allergenicity Rules based on Sequence Homology and exact match for 6 contiguous amino acids, several sequence matches were identified which correspond to allergen sequence matches. Oxitec, however, did not provide sequences for their second generation mosquitoes, OX5034, in subsequent documents provided to the EPA. Novel allergens are a significantly greater concern than already occurring allergens as residents of an area can become desensitized to allergens over time. As humans in the test area have not been exposed to these novel proteins it is expected that any allergic reactions would be more severe.

5. OX5034 may increase antibiotic resistant bacteria.

In the rearing of these mosquitoes Oxitec must use the antibiotic tetracycline. The waste from this tetracycline has the potential to increase antibiotic resistant pathogens.(18). This antibiotic is used to treat MRSA and its non-medical use may lead to tetracycline resistant MRSA.(19). Tetracycline is also used in the treatment of canine heartworm(20) which is transmitted by Aedes aegypti. Testing on another genetically modified mosquito, also reared in tetracycline, found antibiotic resistant bacteria.(21) This is such a concern that many physicians in the Florida Keys have signed a petition(22) calling for a halt to the experimental use of genetically modified mosquitoes in the Florida Keys until testing for antibiotic resistant bacteria has been conducted and the results evaluated for the risk to human health.

6. Tetracycline in the environment may cause 100% of OX5034 to survive instead of 50%

OX5034 mosquitoes are reared in tetracycline contaminated conditions which allows female offspring to survive. Tetracycline is found in pet foods, sewage, drinking water, animal feces, liquid manure and tetracycline is also applied as a veterinary medicine in the water bowls of livestock and pets placed outdoors. Studies suggest Aedes aegypti do not prefer clean water(23) and their larvae can develop in septic tanks, sewage treatment plants and cesspits in Florida(24), and other places where tetracycline may be present.(25) There is even some evidence that Aedes aegypti exposed to antibiotics, such as tetracycline, may increase the transmission of dengue fever.(26). Oxitec's second generation females could also persist in the environment if the genetic killing mechanism evolves(27) over time through genetic mutations allowing both male and female offspring to survive to adults.

7. OX5034 mosquitoes may cause another mosquito that spreads diseases to move in.

Current control methods in Florida, such as the use of the larvicide Vectobac - Bacillus thuringiensis israelensis, often target both Aedes aegypti and Aedes albopictus(28). However, the OX5034 mosquitoes would target only Aedes aegypti, possibly causing a reduction in only Aedes aegypti which could create an opportunity for Aedes albopictus(29) to enter an area and establish itself(30). This could create a situation where Aedes aegypti and Aedes albopictus co-occur. Aedes aegypti and Aedes albopictus already co-occur in some suburban areas of South Florida making this likely for the Keys in this scenario. The Aedes albopictus also transmits mosquito-borne diseases such as dengue fever and chikungunya(31), as well as Zika(32). Aedes albopictus infected with dengue have been found in North America(33) as well West Nile Virus(34), eastern equine encephalomyelitis, Cache Valley(35) and La Crosse virus(36) virus. So, by reducing only the Aedes aegypti population it can create a scenario where there are two different species which spread the same diseases instead of just one. In some cases Aedes albopictus acted as the major vector of both dengue and chikungunya even though both Aedes aegypti and Aedes albopictus were present.(37). Therefore, outbreaks can occur which are caused by Aedes albopictus which do not involve Aedes aegypti and vice versa. At least 16 allergens have been found in Aedes albopictus saliva(38). If Aedes albopictus are able to establish in an area because of a reduction in Aedes aegypti this could increase the number of allergic reactions. Since most people are bitten by mosquitoes in or around their

home, people in some areas are not likely to have been exposed to Aedes albopictus. Therefore natural desensitization likely does not exist among those residents. Furthermore, due to the residents' low or absent natural immunity, having no previous exposure to Aedes albopictus, they are at an increased risk of severe reactions to mosquito bites and this is especially true for young children. The reactions could include large local swellings and redness, generalized urticaria, angioedema, nausea, dizziness, headaches, lethargy and systemic anaphylaxis.

8. OX5034 may be toxic.

Oxitec's second generation mosquitoes have the novel proteins tTA and DsRed2. There is evidence that the tTA protein may be a toxin(39) and neurotoxin(40) to rodents and therefore may be toxic to other mammals such as bats or even humans that might consume them. Some mice studies have detected adverse effects on the lungs(41). Oxitec only mentioned studies using guppies and crayfish exposed to the second generation mosquitoes in their EPA documents. These toxicity studies were short-term and were likely limited to mortality, appearance, size, and behavior. They did not appear to include examination of all major organ systems, histological examination of organs or other health parameters typical of toxicity studies. Multigenerational exposure, as well as transgenerational effects also do not appear to have been considered even though a large number of environmental factors have been shown to promote the epigenetic transgenerational inheritance of disease or phenotypic variation in a variety of different species, including humans(42) Current control methods do not include the potential consumption of synthetic DNA sequences or novel proteins, as found in Oxitec's mosquito, which may be toxic, as a synthetic biological agent, which presents a new risk.

9. Animals consuming OX5034 may alter their microbiota.

Studies have observed transgenes, from the consumption of genetically modified organisms, may transfer to the intestinal microbiota of humans.(43). In animal studies where animals have consumed genetically modified organisms it was observed that some transgenes were able to transform oral bacteria(44). Some transgenes survive passage through the small intestine(45) and have been detected in feces(46). Animal studies have observed transgenes in blood, liver, brain(47), muscle(48), meat and milk of animals fed genetically modified organisms as well as their offspring. Current control methods do not release mosquitoes with synthetic DNA which could be transferred to intestinal microbiota of animals. It may be possible that the DNA will transfer to the intestinal bacteria of insectivores, or humans that accidentally consume OX5034, or animals that consume insectivores that ate the mosquitoes, such as people eating the fish that ate the mosquito larvae.

10. OX5034 will not likely remain in the test area.

Studies have observed Aedes aegypti often traveling up to 800 meters(49), as much as 1000 meters across water(50) and up to 2,500 meters in some cases. In the 1960's the U.S. military demonstrated that when coupled with ocean winds Aedes aegypti could travel up to 3-1/2 miles to shore(51). This distance could easily place a genetically engineered mosquito in a vehicle

intended for another state or another country. Mosquitoes are believed to frequently travel long distances via boat, automobile, etc.(52) and this is also likely true for Florida(53). It is believed that the recent presence of Aedes aegypti in California was caused by commerce via air, railroad, or trucks traveling from the southern U.S.(54) Even Aedes aegypti found as far away as the Netherlands are believed to have traveled there in airplane tires arriving from southern Florida(55). All of these studies point to the very likely possibility of the OX5034 traveling outside of the test area. If the OX5034 escaped the test area this would make ending the experiment nearly impossible.

A better and safer alternative.

OX5034 is not necessary, as a different technology functions the same way as OX5034 and has increased benefits without the same risks as OX5034. Wolbachia-infected mosquitoes inhibit replication of Zika, dengue(56), chikungunya, yellow fever, malaria parasites and filarial nematodes(57). Unlike OX5034, Wolbachia-infected mosquitoes can reduce the population(58) of mosquitoes without the risk of increasing mosquito-borne diseases even if female mosquitoes were accidentally released. Since Wolbachia is a naturally occurring bacterium found in many other insects it is not novel and therefore does not carry the risks of novel allergens. Insectivores in the Florida Keys would also have already been exposed to Wolbachia through other insects. Wolbachia-infected mosquitoes do not require tetracycline in the rearing process and therefore do not increase the risk of antibiotic resistant bacteria in the environment. If Aedes albopictus were to move into the niche habitat as a result of population reduction during the use of Wolbachia, it would not create a scenario of two vectors instead of one because Wolbachia-infected mosquitoes are largely unable to transmit mosquito-borne diseases since Wolbachia inhibits the replication of the viruses involved. In fact, Wolbachia infected Aedes aegypti have already reduced dengue fever by 96% in Australia.(59)

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