GeneWatch UK response to the EPA's consultation on an application 93167-EUP-2 from Oxitec (Docket No. EPA-HQ-OPP-2019-0274)

September 2021

In the application (3167–EUP–2[1]), Oxitec Ltd. requests an amendment and extension to the experimental use permit (EUP) for release of its genetically engineered (GE) OX5034 *Aedes aegypti* mosquitoes expressing tetracycline Trans-Activator Variant (tTAV–OX5034) protein and a fluorescent marker.

Oxitec Ltd. is proposing to:

(i) extend releases of its GE mosquitoes for 2 years in the state of Florida in up to 6,240 total acres at a maximum rate equivalent to 20,000 male OX5034 mosquitoes, per acre, per week;

(ii) expand experimental releases of its GE mosquitoes to the state of California on up to 84,600 total acres at a maximum rate of 30,000 male OX5034 mosquitoes, per acre, per week.

The company's application states that the proposed open release experiments are to evaluate the efficacy of OX5034 mosquitoes as a tool for suppression of wild *Aedes aegypti* mosquito populations. The application also states that female offspring of the OX5034 mosquitoes in the environment die before they mature into adults and therefore exposure to biting female mosquitoes is not anticipated. Both these claims are questioned in the following response.

GeneWatch UK objected to the original Experimental Use Permit (EUP) issued in April 2020 and the issues we raised then remain valid.[2] However, we note that the environmental risk assessment and other documents were not published until this earlier consultation (based on only 2 pages of information from Oxitec) was complete. This means that more information (nine more documents, including a 57-page risk assessment[3]) is now available regarding the original EUP, which raises additional questions and concerns. Secondly, we note that no new information has been provided regarding potential impacts on the environment of California. This is a serious omission, as it means that potential environmental impacts, which could lead to negative impacts on human and/or animal health, have not been properly considered.

Relevant issues are considered further below. A number of references are made to specific pages in the earlier published risk assessment EPA-HQ-OPP-2019-0274-0359[4] and the EPA's Response to Comments made during this earlier consultation, EPA-HQ-OPP-2019-0274-0355[5].

1. Is efficacy really being evaluated?

As noted above, the company's application states that the proposed experiments are to evaluate the efficacy of OX5034 mosquitoes as a tool for suppression of wild *Aedes aegypti* mosquito populations.

First, it is important to note that no assessment is planned of the impact on risk of disease. The EPA states, ""*…because the OX5034 mosquitoes are intended for suppression of Ae. aegypti mosquito populations and are not intended to directly influence disease transmission, epidemiological studies assessing effects on disease transmission are not required…*" (p.106, EPA-HQ-OPP-2019-0274-0355). However, there is a complex relationship between mosquito numbers and disease transmission and evidence of population suppression is not sufficient to show a reduction in disease, or risk of disease.

Second, it is not clear that the applicant has any intention of measuring population suppression of wild *Aedes aegypti* mosquitoes (let alone competitor species, see Section 4.3), nor that the EPA intends to evaluate this. "*Mortality rates will be evaluated by comparing rates of survival to adulthood between treated female larval progeny (those fathered by OX5034 males) and untreated female larval progeny (those fathered by wild males)… Mating fraction data will also be collected under the proposed field trial protocol, as requested by EPA, but as described here and elsewhere, efficacy is most appropriately defined by the effect (percentage mortality) of the pesticide on treated individuals, compared to untreated individuals*" (Oxitec's trial protocol, p. 4, EPA-HQ-OPP-2019-0274-0358). And further, "*Efficacy data analyses are subject to ongoing discussions with EPA*" (Oxitec's trial protocol, p. 21, EPA-HQ-OPP-2019-0274-0358). This means that it is Oxitec's intention merely to show that the progeny of its released GE mosquitoes have lower survival rates than non-GE mosquitoes. This is not sufficient to show any suppression effect of the wild adult female *Aedes aegypti* population (which bite and spread disease). Further, even if mating fraction is calculated, this also does not demonstrate that the population density of biting adult female mosquitoes has been reduced.

It is particularly important to consider this because Oxitec's claims of past success have been highly misleading. For example, Freedom of Information requests to the Cayman Islands' Mosquito Research and Control Unit revealed comments from scientists with access to the data such as:

- "*Whilst Oxitec and MRCU are making public statements proclaiming major reductions in the Aedes aegypti population in the treatment area the data I have seen does not support this*."
- "*To date all the measures recorded have shown no significant reduction in the abundance of Aedes aegypti in the release area*."[6]

In reality, Oxitec's proposed experimental releases will not show whether its GE mosquitoes really make a difference to disease, or even if they will reduce the numbers of the target wild mosquito (*Aedes aegypti*). Thus, there is no benefit to the proposed experiments and hence the risks discussed below cannot be justified.

2. Lack of fully informed consent

It is widely recognised internationally that releases of GE mosquitoes require a process of fully informed consent. For example, Macer (2005) states, "*Information should be exchanged as broadly as possible with community leaders, members of the local community, and the mass media. Consent should be obtained from the communities involved. Specific*

mechanisms to obtain individual and group consent need to be developed for public health interventions".[7]

In contrast, the EPA states, "*Oxitec is not required under EPA's human studies rule to obtain informed consent of those living in the areas where the Oxitec mosquitoes would be released…*" (EPA-HQ-OPP-2019-0274, p. 137). This is based on the finding that, "…*the research involved with Oxitec's release of mosquitoes does not meet the regulatory definition of research involving human subjects. Because the proposed information to be collected as part of this research does not involve human subjects, it is not necessary to evaluate whether the research would constitute intentional exposure of human subjects*".

However, human subjects will be living in the release areas. They may be bitten by surviving adult female GE mosquitoes, swallow male or female GE mosquitoes, and their risk of mosquito-borne diseases could increase if wild mosquitoes move to a different area, or there is a rebound of *Aedes aegypti* mosquitoes following the releases, or if the competitor species *Aedes albopictus* moves into the area. It is illogical to require consent only if these potential adverse impacts on humans are monitored and assessed.

The EPA's view that consent is not required is therefore ethically and legally extremely questionable.

3. Missing information

Some sections of the risk assessment (EPA-HQ-OPP-2019-0274-0359) remain blacked out as Confidential Business Information (CBI): one line on p. 18 (mosquito rearing); one line on p.21 (fecundity); 2 lines on p.22 (longevity); 15 lines on p. 28 (about the allergic potential of the fluorescent protein DsRed2-OX5034). The section on tetracycline in the environment (p.32) does not say what levels are required for female OX5034 mosquitoes to survive to adulthood. The risk assessment also refers to numerous Oxitec studies which are not public.

The EPA's 'Response to Comments' (p.132) also refers to CDC advice: however, the full advice has not been provided (this is discussed further in Section 4.1, below).

No information regarding populations of *Aedes aegypti* in California has been provided, nor any evidence regarding competitor species or predators/prey. This makes it impossible to assess environmental risks.

There is also no published information regarding Oxitec's earlier releases of OX5034 GE mosquitoes in Brazil.

Some specific omissions of data on specific issues are highlighted further below. A list of further studies that would be required is also included in our previous submission.[8]

4. Inadequate consideration of risks

GeneWatch UK has previously drawn attention to a number of risks that have been not been adequately considered, including[9]:

- potential release of biting female GE *Aedes aegypti* mosquitoes;
- potential release of infected mosquitoes;
- survival and spread of GE mosquitoes;
- use of antibiotics to feed the GE mosquitoes;
- use of a non-native strain of the *Aedes aegypti* mosquito;
- complex mosquito population responses;
- adverse effects on the environment.

Rather than repeat the same evidence in detail again, we highlight new evidence (and gaps in evidence) in relation to some specific issues, including from the risk assessment published after the earlier consultation, and in relation to proposed releases in a new state (California).

4.1 Release of a non-native (Mexican) strain of Aedes aegypti into the environment

The risk assessment provides the background of the strain (which was published for the first time after the previous consultation): "*The OX5034 Ae. aegypti line was developed in 2013 by transformation of a "Latin American Ae. aegypti wild-type" strain (LWT) with the vector pOX5034. The strain was subsequently backcrossed several times to obtain the OX5034 homozygous Ae. aegypti for which the EUP is sought. The background of the LWT strain is comprised of genetics from ten separate Ae. aegypti colonies. These colonies were established from mosquitoes that were collected in the Mexican State of Chiapas in 2006 (Wise de Valdez et al. 2011)*" (p.10, EPA-HQ-OPP-2019-0274-0359).

It remains unclear why the EPA considers it lawful or safe to release a Mexican strain of *Aedes aegypti* mosquito in the USA without even testing its vectorial capacity. Introducing an Aedes aegypti strain with increased vectorial capacity for any disease (dengue, zika, chikungunya) could have serious and irreversible repercussions for public health.

Evans et al. (2019) highlights the spread of genes from Oxitec's previous strain of GE mosquito, OX513A, into the wild population from previous experiments in Brazil.[10] The EPA's 'Response to Comments' (p.132) states, "*EPA has, however, for this EUP, sought the advice of technical experts at the US Centers for Disease Control and Prevention (CDC) on several of the technical issues presented by OX5034 release, including their analysis of the recent publication by Evans et al. The CDC advice has been incorporated into the decision reached by the Agency on the request for an EUP to field test OX5034 mosquito*". The EPA states (p.140) that this question is not related to the CDC import permit. However, this is questionable because the import permit is what prevents the release of this imported non-native mosquito strain into the environment. Such strains (whether or not they are genetically engineered) are usually required to be studied only in contained use, due to the risks highlighted in the Evans et al. paper, particularly the risk of increasing the capacity of wild *Aedes aegypti* mosquitoes to spread disease, by introducing genetic changes not present in the local mosquito population. This risk is likely to be much higher with the new OX5034 strain than with the OX513A strain studies in Brazil, as discussed below, because the new strain is female-killing only.

The risk assessment (EPA-HQ-OPP-2019-0274-0359) also refers to joint work on this issue with CDC (p.40) and cites this memo: USEPA. 2020. Summary of the Data and Information Related to Vectorial Capacity Presented for the New Product OX5034 (EPA File Symbol: 93167-EUP-E). Memo from Amanda A. Pierce to Eric W. Bohnenblust, dated February 12, 2020. This may be the document on vectorial capacity that has now been published as EPA-HQ-OPP-2019-0274-0351.[11] However, this is just a "summary' and any full consideration of this issue (if it has been undertaken) has still not been published for public scrutiny. It is worth noting that this document recognises the importance of this issue, stating, "*the degree of introgression is likely to be significantly higher than that of the OX513A strain due to higher larval survival rates (approx. 5% in OX513A versus 50% in OX5034)…Traits associated with a disease vectoring species such as Ae. aegypti that may carry risk if introgressed into a wild population are likely to be linked to vectorial capacity, including vector competence, fecundity, and longevity*" (EPA-HQ-OPP-2019-0274-0351). Although this document concludes that, "*it is not expected that introgression of OX5034 strain genetics would increase the vector competence of the wild mosquito population*", no evidence is provided in the document to support such a conclusion. Further, the EPA's subsequent response to comments goes much further than this document when it states (p. 31) that the risk is "*negligible*". It is hard to see how this risk can have been properly assessed without testing the vectorial capacity of the introduced strain and any potential admixed strains. Even had such testing taken place, the legal basis on which a non-native strain of mosquito can be released into the environment remains unclear (as highlighted in our previous submission[12]).

4.2 Failure to test toxicity and allergenicity and the potential for females to survive the killing mechanism

The risk assessment states, "*No determination of the allergic or toxic potential for tTAV-OX5034 and DsRed2-OX5034 has been made at this time*" (p.24, EPA-HQ-OPP-2019-0274-0359).

However, the EPA has accepted a waiver request on the grounds that exposure will be negligible: "*The information provided on the hazard assessment of tTAV-OX5034 and DsRed2-OX5034 is, on its own, inadequate to support the waiver requests. However, exposure to OX5034, tTAV-OX5034, DsRed2-OX5034 and the genetic material encoding them through the dermal, oral, pulmonary, and ocular routes of exposure is expected to be negligible. Therefore, overall the waiver requests for the acute oral toxicity, acute inhalation toxicity, acute dermal toxicity, primary eye irritation, and primary dermal irritation are acceptable*" (p. 23/24, EPA-HQ-OPP-2019-0274-0359). Part of this section on allergenicity is blacked out.

However, even if GE females are not released, the finding on lack of exposure to biting females depends on the assumption that females do not survive to adulthood. In reality, survival may occur if resistance develops or because of environmental exposure to tetracycline (see more detail in our previous submission[13]). Ingestion may also be a potential exposure route for humans or animals, as females are expected to die at the larval stage in the water where they breed. Further, GE males are expected to be released in large numbers and to survive in each generation and could be swallowed by humans or animals.

In fact, the EPA has not assessed whether resistance will develop and therefore has made no assessment of whether GE females will survive in the longer term. It states in the Response to Comments: "*Should EPA receive a request for registration of OX5034 under FIFRA section 3, EPA has the option at that time to consider whether a program designed to manage the potential for resistance to emerge in OX5034 is feasible and warranted*" (p.16, EPA-HQ-OPP-2019-0274-0355). Thus, there is no basis to conclude that exposure to biting females will be low. In addition to the information in our previous submission, a new reference in relation to the development of resistance should be taken into consideration.[14] This paper adds important evidence to support the view that evaluation of resistance should be done (on a large scale) in contained use, before any release.

In relation to female survival due to exposure to tetracycline (which can switch off the killing mechanism), there are also important gaps in evidence. The EPA states that "*most*" septic tanks in Florida are now gone (Response to Comments, p. 45) and other sources of tetracycline (e.g. cat feed, animal waste) not plausible (Response to Comments, p.46). However, concerns about potential exposure to tetracycline are sufficient for conditions to have been applied to the Florida trial in an attempt to limit such exposure – for example, Oxitec's Protocol (EPA-HQ-OPP-2019-0274-0358) states that, "*The outer boundary of the trial area (denoted by the traps furthest from the central release point) will be greater than 500 m from commercial citrus growing areas and from sewage treatment plants*". It is a matter of serious concern that no information has been supplied regarding potential tetracycline exposure at or near proposed release sites, particularly in the new proposed sites in California, including in commercial citrus growing areas or in septic tanks.

Additional complexity needs to be considered in the light of recent studies showing that mosquito microbiomes (which can be influenced inter-generationally by the use of antibiotics) can influence vectorial capacity.[15,16]

The EPA has concluded that OX5034 "*is not expected to establish within the test area*" (p.94, Response to Comments). However, this conclusion seems to rely on a caged trial to argue the transgene will not persist (p.38 to 39): this does not make sense because the caged population collapses completely, which would not happen in the real world, where wild mosquitoes are mobile and can move elsewhere. Further, in a caged trial the GE mosquitoes will not be exposed to tetracycline and the duration of the trial may not be sufficient to allow resistance to develop,

4.3 Complex mosquito population dynamics: impacts on health and biodiversity

The risk assessment argues that no species rely on *Aedes aegypi* if the population falls (p.43-49), but it does not address the impacts on human health or biodiversity of fluctuations (high numbers during releases, followed by potential reductions and then perhaps a rebound in population numbers), or movement of wild mosquitoes from one area to another in response to the releases (as appears to have happened in the Cayman Islands and elsewhere in response to earlier releases of the OX513A strain).

Further, there is no analysis of potential response of the competitor species *Aedes albopictus* (which is also an important disease vector) to fluctuations in *Aedes aegypi*, despite concerns that suppression of *Aedes aegypti* (if successful, even temporarily) could lead to more *Aedes albopictus* moving into an area, due to reduced competition. This issue is discussed in more detail in our previous submission.[17] As Oxitec's former Chief Scientific Officer, Luke Alphey has stated, "*Since* Aedes aegypti *and* Aedes albopictus *are known to compete … it is possible that the successful implementation of* …[GE mosquito] *gene drives could lead an existing* Ae. aegypti *population to be displaced by* Ae. albopictus *where it would not otherwise have been. This would likely hamper efforts to eliminate viruses such as dengue since* Ae. albopictus *are also competent vectors*...".[18] Both species are present in California[19] (as has been previously noted for Florida[20]), yet no information is provided in the application regarding any surveys of mosquito species in the proposed release areas.

4.4 Lack of feeding trials

No feeding trials have been done for mammals or birds (risk assessment, p.45-46), although Oxitec has undertaken some feeding trials for an 'aquatic invertebrate' (p.46, risk assessment) and with crayfish and guppies (p.48, risk assessment).

In addition, there is no information regarding local mosquito predators or prey at any of the sites. This is a particularly striking omission in the light of the proposal to undertake open releases of GE mosquitoes in a new state (California).

5. Conclusions

In summary, considerable further evidence is needed to assess whether the use of the pesticide under the proposed permit (including its method of delivery via GE mosquitoes) may cause unreasonable adverse effects on the environment. As well as considering the legal obligations highlighted above, the EPA should therefore specify that further studies be conducted before publishing a full EIS for public consultation.

References

[1] file:///Users/helenwallace/Downloads/EPA-HQ-OPP-2019-0274-0363_content.pdf

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[3] https://www.regulations.gov/docketBrowser?rpp=25&so=DESC&sb=commentDueDate&po=0&dct=SR&D=EPA-HQ-OPP-2019-0274

[4] Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034;New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti. ID: EPA-HQ-OPP-2019-0274-0359. https://www.regulations.gov/document/EPA-HQ-OPP-2019-0274-0359

[5] Response to Comments OX5034 to the Notice of Receipt of an Application for an Experimental Use Permit Number 93167-EUP-E. ID: EPA-HQ-OPP-2019-0274-0355. https://www.regulations.gov/document/EPA-HQ-OPP-2019-0274-0355

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[11] Memorandum: Summary of the Data and Information Related to Vectorial Capacity Presented for the New Product OX5034 (EPA File Symbol: 93167-EUP-E) Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034), a Variant of the Modified Discosoma spp. DsRed2 Protein, and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 Aedes aegypti... ID: EPA-HQ-OPP-2019-0274-0351. https://www.regulations.gov/document/EPA-HQ-OPP-2019-0274-0351

[12] GeneWatch UK comments on EPA-HQ-OPP-2017-0756: Application 93167-EUP-R from Oxitec Ltd. 27th March 2018. http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/GeneWatch_EPA_Oxitec_consul_fin.pdf

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