# Oxitec's Genetically Modified Mosquitoes: Failing in the field?

GeneWatch UK

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The UK company Oxitec, now owned by US company Intrexon, first conducted experimental open releases of genetically modified (GM) mosquitoes in the Cayman Islands in 2008-09. Experimental releases re-started in the Cayman Islands in 2016 and there are plans to expand these releases to a larger scale roll-out in 2017.[1,2] This briefing updates an earlier briefing by GeneWatch UK on the proposed roll out, published in March 2017.[3]

A significant expansion of GM mosquito releases raises important questions about cost-effectiveness, scientific protocols, risk assessment and public consent.

This briefing summarises the questions and concerns about these plans. New information, obtained since March 2017, is discussed in an addendum at the start of this updated briefing. This new information, particularly from an annual report released after approval of the roll out by the National Conservation Committee (NCC), raises concerns about spikes in numbers of adult female mosquitoes as a result of the releases, and the inadvertent release of large numbers of female GM mosquitoes (which may bite and transmit disease). In addition, claims of efficacy are shown to be misleading.

Key questions are:

- Is this technology effective?
- How much would it cost and is it cost effective?
- Are the Cayman Islands' regulations adequate?
- Have alternatives been properly considered?

**Addendum**

Further information about the completed (phase 1) and proposed (phase 2) releases has been published following the June 2017 meeting of the National Conservation Council (NCC).[4] This information includes:

- Two interim reports on the releases of GM mosquitoes made in 2016 and early 2017: one from October 2016[5] and one from February 2017[6];
- Applications/permits relating the releases made to June 2017: (i) application to import genetically modified mosquitoes, beginning June 2016 (date stamped to indicate permission given on 8th June 2016)[7]; (ii) application/permit to import genetically modified mosquitoes, Nov 2016 to June 2017 (date stamped 10th November 2016)[8];
- Two corresponding permits for import of GM mosquitoes: (i) the original permit valid from 20th June 2016 to 30th June 2017, for import of 150 million GM mosquito eggs (1.64kg), over a 12 month period: (ii) a variation to the permit valid from 10th November 2016 to 10th June 2017, allowing up to an additional 1kg of eggs to be imported and pupae to be "contained within sealed release devices" at the MRCU insectary[9];
- The Mosquito Research and Control Unit's (MRCU's) submission to the NCC for a further "expansion phase" of open releases[10];

- The application for the proposed expansion phase, allowing import of up to 2kg of eggs per month (not date stamped at the time of publication i.e. permit unissued at that time)[11]; and
- The Department of Environment's "screening evaluation" of the risks of the expansion proposal.[12]

A version of the National Conservation Council (NCC) Guidance Note on Risk Assessment of alien and genetically altered species is also now publicly available.[13] However, there has been no public consultation on this guidance.

In addition, an MRCU annual report on the "Friendly *Aedes aegypti* project in West Bay", published in June 2017 and covering the releases up to 25th June 2017, has been released in response to a Freedom of Information request.[14]

***The new documents confirm poor efficacy of Oxitec's technology***

In relation to the now completed (phase 1) releases, at public meetings in May 2016, Oxitec stated that between 100,000 and 200,000 GM male mosquitoes would be released each week in the target area in West Bay for several weeks and that, after two to three months, the population of the *Aedes aegypti* mosquitoes was expected to fall dramatically.[15]

However, the February 2017 interim report shows that Oxitec did not manage population suppression until the dry season (early 2017), when the population had already dropped significantly, and to get the observed effect the MRCU had to first spray as well. Releases were increased to 500,000 to achieve any observable effect (as measured using egg traps). They claim 79% population suppression (based on egg traps) but as with earlier experiments, there is no clear baseline or measurement of adult female numbers (discussed further below). The existence of the November 2016 permit variation, which allows pupae to be "contained within sealed release devices" at the MRCU insectary may reflect the need to release greater numbers of adults, or might suggest the company may have resorted to releasing pupae from release devices, to top up the adult releases (as they did in earlier experiments). Releases of pupae, if they occurred, could be a means to get around the restriction on adult male releases, without providing further public information.

Minutes of the NCC's discussion of the original permit for releases[16] have been published which confirm that conditions agreed for the original permit include that not more than 22 million adult male mosquitoes may be released during the course of the project (page 3 of the minutes from the NCC's 18th May 2016 meeting). This limit remained unchanged when the permit for additional eggs was approved on 10th November 2016, however the minutes signed off the following March[17] merely state that the MRCU had been sent a copy of the decision and that the permit is attached. No in-depth discussion of the permit variation by the NCC has been recorded.

Documents from a "special meeting" of the NCC, held on 3rd May 2017, reveal a further application made on 25th April 2017 (pages 3 to 7), to import an additional 500 grams of eggs as part of phase 1 of the project (before the roll out was approved).[18] There is no reference in the application to any restriction on numbers to be released. There is also no record in the minutes of the meeting (released as a result of a Freedom of Information request), as to whether this application was discussed or approved. However, the existence of the application would suggest that Oxitec has not succeeded in maintaining population suppression using the number of GM mosquitoes originally suggested, and was looking to significantly increase release numbers again.

A report of the phase 1 project is required to be provided by July 30th, 2017 under the existing DoE permit, with an additional project conclusion report at a later date if necessary (page 13 of the "Expansion phase" document). However, the NCC did not wait for this phase 1 report before issuing a permit for phase 2 (including an island-wide roll out) on 14th June 2017. An annual report on the project by the MRCU, covering results up to June 25th 2017, has since been released as a result of a Freedom of Information request. This report states that a total of 3136g of GM mosquito eggs had been imported by 22nd May 2017, out of 3140g permitted. Therefore the additional application submitted to the NCC on 3rd of May would have had to be approved for phase 1 releases to have continued to the end of July. The report also highlights problems with egg quality, which has led to storage conditions being improved in both the UK and Grand Cayman.

More importantly, the MRCU annual report reveals problems with the release of adult female GM mosquitoes (which bite and may transmit disease, discussed further below) and spikes in the adult female mosquito population as a result of the releases, both of which may pose risks that have not been fully considered. For the first time, female adult mosquito numbers collected from traps are included in the published data (rather than just egg traps) (Figure 1B). These show, as discussed in the text (page 6), that no adult mosquito suppression was observed until February (week 7) in 2017, and that the egg traps therefore provide a poor measure of success in supressing the number of adult female mosquitoes (which bite and transmit disease): a concern that has previously been raised in the scientific literature, and which may invalidate claims of success elsewhere, which are all based on data from egg traps.[19] Further, the graph shows significant increases (spikes) in adult female mosquito numbers (green line) in the release area 5 to 7 weeks after the releases begin, and again 7 to 8 weeks after the releases are increased. These spikes in the adult female population exceed 150% of the comparator population, but their true extent is not shown as the peaks are cut off on the graph. There is no discussion of these spikes in the text, and no consideration of the implications of the increased numbers of adult female mosquitoes for the risk to health of the local population. No explanation for the spikes is given and the absolute number of females is not given, nor whether they are GM or non-GM females. One possible cause could be that the spikes are caused by the inadvertent release of GM females (see further below). Or, female GM mosquito larvae, produced as a result of matings between released GM males and wild females, might have unexpectedly high survival rates. Alternatively, more wild female mosquitoes might have flown in from surrounding areas to mate with the released males.

In addition, the graph reveals that the experiments did not continue long enough to see if the suppression shown in the tail of the graph continues into the wet season. This is critical to establish whether suppression is continued in the longer term, or whether increased releases, with likely further spikes in the adult female population, will be needed in the future.

The expansion proposal relies heavily on the releases being able to achieve suppression so the production capacity can be moved around once treated areas are in a "maintenance phase". In "stage 1" of the phase 2 roll-out (this year) the plan assumes there would not be any new production capacity (current capacity is up to 700,000 GM males per week) but it would be possible to either expand the area of releases in West Bay, or make releases on Cayman Brac, because the releases needed in the "maintenance phase" of the existing West Bay area would be reduced due to suppression having already been achieved. However, this is very dependent on the idea that suppression is achieved and can then be maintained with a lower level of releases. For "stage 2" (roll out over 18 to 24 months, starting in 2018) production capacity would be increased (up to 10 million GM males per week), to do releases across the whole of Grand Cayman. This might be done with more mobile labs, or by converting a warehouse. But this plan is still very dependent on the claims

regarding population suppression being achievable, as the release numbers would be kept within production capacity by only treating inhabited areas and moving the releases around as each area enters a maintenance phase. When the whole island is in "maintenance", the plan is for MRCU to take over from Oxitec to treat only localised “hotspots” or re-entry points (p. 9 of "Expansion phase" doc). However, based on the data released so far, this seems very unlikely to be achievable.

***The new documents confirm an inadequate basis for approval of the roll out and increase concerns regarding risks***

Approval for the proposed island-wide roll out of releases was reportedly given by the National Conservation Council (NCC) on 14th June 2017 with some conditions, including the approval of the Medical Officer of Health.[20] However, the contract between Oxitec and the Cayman Islands Government (referred to on p. 9 of "Expansion phase" document[21]), has apparently not yet been signed. According to the “Expansion phase” plan, a formal agreement between Oxitec Cayman Limited and the Government of the Cayman Islands is anticipated, to establish the main program activities and responsibilities for each party, including a date by which “significant suppression” of the local population of *Aedes aegypti* throughout the island of Grand Cayman should be achieved. It is hard to see how such a commitment can be made, given the problems with population suppression identified above.

There is no new information on biosafety issues in the application or permit for expansion, as the applicants say that these were already dealt with in the first application for release. Thus, there has been no attempt to provide further information to answer any of the questions about risks. However, the documents do contain further information about the very limited efficacy of Oxitec’s approach, including significant spikes in the numbers of adult females in the release area, which could pose a risk to health (as noted above). In addition, the annual report on the project by the MRCU, covering results up to June 25th 2017, reports a problem with sorting the male and female GM mosquitoes, which led to increased releases of GM females (which bite and can transmit disease). The report reveals that a sorting criterion of 2 or fewer females per batch of 1,000 pupae had been set, but checks by MRCU on one production batch on May 12th 2017 revealed 9 females in one release pot (of 500), nine times the agreed level. The report claims that additional measures have now been put in place to ensure that no more than 2 females are released per 1,000 mosquitoes. At the peak release rate in phase 1 of 500,000 mosquitoes per week, this nevertheless means that 1,000 biting females a week could have been released even if the sorting criterion was met, with up to 9,000 GM females released a week if the problem later revealed by the MRCU check was occurring during the peak releases. This is a serious problem, which does not seem to have been discussed or addressed during the NCC’s June meeting, when the annual report was not available. During the proposed phase 2 releases (island-wide roll out), many more biting female GM mosquitoes could be released. The increased production capacity (of up to 10 million GM males per week) would result in release of up to 20,000 biting GM females being released a week if the sorting criterion is met, or 180,000 biting GM females being released a week if 9 females were released per pot.

The Department of Environment’s “screening evaluation” was produced before the release of this report and does not address this new information. It relies heavily on the existence of risk assessments already made in other countries and recommends “*that the Council be guided primarily by the independent risk assessments referenced below, recognizing these are independent of the applicant and have considerable relevance to the current application*”. However, none of these consider the new evidence produced as a result of phase 1 of the experiments in the Cayman Islands. Further, the cited risk assessments published in the USA and Malaysia cover only experimental releases – not a full scale roll

out – and have both been withdrawn, as the related experiments did not go ahead at all (in the case of the USA), or beyond the first limited release (to test flying distance) in Malaysia. The risk assessment published in Brazil includes a Dissenting Opinion from two experts[22], raising significant concerns, and commercial releases there have yet to be approved by Brazilian health authority. The previous risk assessments in the Cayman Islands do not meet international standards and do not take account of the new information contained in the MRCU annual report and other documents. Further, they do not account for the proposed scale-up of the releases.

The minutes of the NCC's June meeting are not available at the time of writing, but the conditions are presumably those recommended by the Department of Environment in its "screening evaluation", namely:
"*a. that a plan for disposal of waste water from all current and future rearing facilities for OX513A in the Cayman Islands is submitted and implemented to the satisfaction of the Water Authority and the Department of Environment, such that no potential exists or can be created for OX513 Aedes aegypti to breed in wastewater containing tetracyclines from these facilities.*
*b. that before any OX513A Aedes aegypti are transported to or released on Cayman Brac or Little Cayman, tests are conducted by the applicant to establish whether OX513A Aedes aegypti are at all capable of hybridizing with Aedes mediovittatus, and that expansion of OX313A Aedes aegypti releases to the Sister Isles be subject to separate consultation with the NCC after completion of these tests*
*c. that OX513A Aedes aegypti eggs transported to the Cayman Islands are produced from females whose blood meals are from synthetic blood only*
*d. that active monitoring for persistence or spread of OX513A Aedes aegypti is continued on a permanent, ongoing basis in and around all release areas and around rearing facilities, and that any anomalous occurrences are reported to the Council immediately and investigated by the applicant as a matter of urgency*
*e. that before decision by the Council, the views of the Chief Medical Officer be sought on this application*".

There is no mention of any measures to investigate or address the spikes in adult females, revealed in the MRCU annual report (which was not published until after the NCC meeting), or the problems with the release of adult GM female mosquitoes (which bite and may transmit disease).

# Oxitec's Genetically Modified Mosquitoes: Ready to roll out?

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## Background

Oxitec's patented technique for genetically modifying insects is known as RIDL (Release of Insects carrying a Dominant Lethal genetic system). All the company's open field experiments to date involve its OX513A strain of the *Aedes aegypti* mosquito, which is genetically engineered to contain a red fluorescent marker and the RIDL 'conditional lethality' trait. The mosquitoes are genetically engineered to die at the larval stage in the absence of the antibiotic tetracycline, which acts as a chemical switch to allow breeding in the laboratory.

Oxitec's male OX513A GM mosquitoes are intended to mate with wild females and produce offspring which die as larvae. Repeated releases of many millions or billions of GM males, vastly outnumbering the wild male mosquito population, are intended to reduce the total adult population of mosquitoes over time, as many of the GM offspring fail to survive to adulthood.

The GM mosquitoes released in the experiments are of the *Aedes aegypti* species, which transmits the tropical diseases dengue fever, zika and chikungunya.

The UK company Oxitec, now owned by US company Intrexon, has conducted experimental open releases of genetically modified (GM) mosquitoes in the Cayman Islands, Malaysia, Brazil and Panama. Oxitec's releases of GM mosquitoes in Panama and Malaysia have ceased, due to concerns about costs, effectiveness and risks. Further proposed trials in the USA (in Key Haven, Florida Keys) have been halted while a new site and new authorisation is sought, following a local vote against the trials and the threat of legal action.[23] The Food and Drug Administration (FDA) authorisation for the Key Haven trials has therefore been withdrawn.[24,25] In Brazil, trials continue in the city of Piracicaba, but commercial releases have yet to be approved by the Brazilian health authority, ANVISA, which wants to see evidence of benefits to health before giving its approval, in line with recommendations from the World Health Organisation (WHO).[26,27,28]

Questions and concerns about open releases of Oxitec's GM mosquitoes are outlined below.

### 1. Is Oxitec's technology effective?

Oxitec has made repeated claims in public materials and press releases that its releases of GM mosquitoes can suppress wild *Aedes aegypti* mosquito populations by 90% or more. For example, the Oxitec and MRCU public leaflet used in the Cayman Islands states "*Each area* [where GM mosquitoes have been released] *achieved a reduction in mosquitoes of more than 90%*" and shows a map with the Cayman Islands (96%), Panama (93%) and three trials in Brazil (Itaberaba, 93%, Mandacaru 99%, Pedra Branca 92%). However, there are a number of serious problems with these claims, which are discussed further below.

Further, there is as yet no evidence from any country that releases of GM mosquitoes can reduce the incidence or harm to health caused by zika, dengue or chikungunya.

No results have yet been published from Oxitec's 2016 trials in Grand Cayman, which reportedly involved the release of more than 6 million male GM mosquitoes.[29]

The results of these trials and the company's previous experiments need to be examined critically, before any decision is made to scale-up and roll out the releases across Grand Cayman. In particular, the following issues need to be considered.

### *1.1.1 Oxitec's claims of reduced mosquito populations are not based on good science*

> "*To verify those numbers we talked to Danilo Carvalho at the University of São Paulo in Brazil, who helped analyse the data. He tells us the numbers are more like 60 to 70% reduction, not 90, and actually called into question Oxitec's methods and said their analysis was below scientific standards*." Phil Torres [journalist], TechKnow, Al Jazeera 29th November 2016 (at 12:54).[30]

During all its experiments, Oxitec has used traps which catch adult males (called BG traps) and egg traps (ovitraps) to try to measure the effects of its releases on the wild population of mosquitoes. However, there are problems with how Oxitec interprets its data and there is no direct evidence of a fall in the population of biting female mosquitos, which transmit disease.

The lead author of Oxitec's paper reporting Oxitec's results from Itaberaba in Brazil is Danilo Carvalho, who was reported by Al Jazeera in November 2016 as questioning Oxitec's claims and methods (cited above).

Only one of Oxitec's published papers include the raw data for their calculations. This is the paper which reports the results from Itaberaba.[31] These results have been the subject of a critique published in the Lancet Global Health.[32] As shown in the Appendix to this paper, Oxitec's claim of 95% adult suppression in this trial is based only on captured adult male, not adult female mosquitoes. This means no conclusions can be drawn about the effect of the releases on the number of adult biting female mosquitoes which transmit disease. The start and end time for the claimed reductions are chosen by Oxitec to give the most favourable results. Further, there is no control area with which to compare the numbers of adult males, although controls are provided for the egg traps.

Insufficient data is provided in any of the papers to draw definitive conclusions about the impacts of the releases on wild mosquito populations. One issue is that wild mosquitoes may simply move to neighbouring areas to mate, once the GM mosquito releases become high enough. In the 2009 Cayman Islands experiments, the number of wild *Aedes aegypti* mosquito eggs, measured using egg traps (ovitraps), was observed to increase in the neighbouring control area as the population in the release area decreased (Figure 2c).[33] The same effect can be seen in Oxitec's experiments in Itaberaba, which compare ovitrap data from the control area with data from adult male traps in the release area (Figure 2D).[34] Oxitec has assumed that the difference between the number of wild eggs counted in control area and the number of eggs or adult males in the release area was caused by the desired population suppression effect where the GM mosquitoes were released. However, it could alternatively mean that more *Aedes aegypti* eggs were laid in the control area bordering the releases because wild males and/or females moved into the control area from the target area to move away from the releases. This would mean the difference between the ovitraps in the control areas and the data from the release areas was caused (wholly or in part) by more eggs being laid in the control area, rather than by mosquitoes in the release area dying off. If so, Oxitec's calculations of a reduction in the wild mosquito population would be incorrect.

In Panama, the experiments were different and compared ovitrap data in three different villages: therefore there are no data from Panama about whether more eggs were laid neighbouring the release site.[35] Although Oxitec has done some further experiments

elsewhere in Brazil, the results of these have not been published. Thus it is impossible to draw definitive conclusions about suppression of the wild population of *Aedes aegypti* mosquitoes from any of Oxitec's published papers. In particular, there is no direct evidence of a fall in the number of biting females.

### *1.1.2 Release ratios of GM to wild mosquitoes are extremely high*

One measure of the success of population suppression is the "release ratio" needed i.e. the ratio of released GM male mosquitoes to wild male mosquitoes. However, Oxitec's published papers do not allow the release ratios to be easily determined. An unpublished report from Oxitec's experiments in Itaberaba, Brazil suggests that release ratios there reached up to 54 to1.[36] More than half a million mosquitoes a week were produced during the late phase of these experiments and the releases were concentrated in a small area of houses less than 500m by 200m.

In its first experiments in the Cayman Islands, Oxitec had to significantly increase its releases of GM mosquitoes, from the expected 3,150 males per hectare per week to about 14,000 per hectare per week, targeted on a small 16 hectare area, in order to achieve the claimed population suppression effect. When local residents complained about the nuisance caused by the very large number of mosquitoes, Oxitec halved the number of adults released and deployed about 5,600 GM pupae in cages spaced 70-90m apart across the site three times a week (see supplementary material to Harris et al., 2012[37]). Oxitec's computer modelling of the results from the 2009 Cayman Islands predicts that that releases of 7 million GM mosquitoes a week, in an initial phase, would be needed to suppress a population of only 20,000 wild mosquitoes (10,000 males), followed by releases of 1.9 million GM mosquitoes a week for long-term suppression, if a mixture of pupal and adult releases are used, or 2.8 million a week if only adults are released.[38] The authors of the paper admit that in the real world, where mosquito populations are more complicated, higher numbers might be needed.

Oxitec has chosen to release its GM mosquitoes under favourable conditions in small areas, which may not apply when the trials are scaled up.

### *1.1.3 It is not clear if a reduction in Aedes aegypti numbers will lead to any benefit to health*

"*GMMs [Genetically Modified Mosquitoes] must be effective in reducing transmission of the targeted pathogen(s) and not detrimental to the environment and human health if they are used as public health intervention tools. Demonstration of efficacy will be a critical determinant for decision-making about deployment*". World Health Organisation, 2014.[39]

"*The trials we've conducted so far are not on a large enough scale to be able to show any sort of dengue control*". Derek Nimmo, product development manager at Oxitec.[40]

"*There is only a loose correlation between a reduced number of mosquitoes and a reduced number of dengue cases*". Dr. Phil Lounibos, University of Florida.[41]

"[Dr. James] *suggested that these genetic tools might not be the best strategies for ZIKV* [zika virus] *given that at this point there seem to be multiple vectors not only at the species but also at the population level. The current genetic technologies would not be appropriately applied to such complex systems*".[42]

Even if suppression of the wild population of *Aedes aegypti* mosquitoes is successful, this might not lead to a reduction in the harm caused by tropical diseases. There are several important issues:

(i) Disease transmission thresholds are not well known and even a small number of mosquitoes may still lead to transmission of disease;
(ii) More than one species can transmit zika, dengue and chikungunya. Non-target species, such as *Aedes albopictus*, will not be reduced by the releases and might possibly increase due to reduced competition, particularly in the longer term;
(iii) There is a complex relationship between human immunity and infection, so in some circumstances reducing infection can lead to reduced immunity and hence a rebound in cases of disease;
(iv) There are several mechanisms through which the technology could become less effective over time (for example, if the GM mosquitoes evolve resistance to the killing mechanism), making a rebound in disease more likely.

There has been no monitoring of the impacts on dengue fever, zika or chikungunya of Oxitec's GM mosquito releases in any country, despite a scientific consensus that assessing impacts on disease is essential to assess the efficacy of new technologies.[43,44] Oxitec and its research partners in Brazil have both admitted that the experiments there (the largest ones conducted) are inadequate to assess the impacts on disease.[45,46]

Oxitec has claimed that the World Health Organisation (WHO)'s Vector Control Advisory Group (VCAG) has "*issued a positive recommendation in support of Oxitec's self-limiting mosquito (OX513A)*".[47] However, the relevant VGAC statement in fact says: "*Full-scale programmatic deployment is not currently recommended for any of the five new potential tools reviewed by VCAG. However, the VCAG recommended the carefully planned pilot deployment under operational conditions of two tools (Wolbachia-based biocontrol and OX513A transgenic mosquitoes) accompanied by rigorous independent monitoring and evaluation*".[48] More specifically the VCAG states that Randomised Controlled Trials (RCTs) "*with epidemiological outcomes should be carried out to build evidence for routine programmatic use of OX513A Aedes against Aedes-borne diseases*". This advice is at odds with Oxitec's claims that its technology is already ready for deployment.

*Aedes albopictus* is another species of mosquito which is found in the Cayman Islands and which can also transmit dengue, chikungunya and zika.[49,50,51,52] Although this species is currently found in relatively small numbers in the Cayman Islands, it is an invasive species which has spread worldwide and has for example competitively displaced *Aedes aegypti* in most of Florida and in Bermuda.[53,54] *Aedes albopictus* has been responsible for concurrent epidemics of dengue and chikungunya in some countries and its presence can also extend the dengue season and perhaps introduce new viruses.[55,56,57,58,59,60] Non-target species, such as *Aedes albopictus*, will not be reduced by releases of GM *Aedes aegypti* and might possibly increase if population suppression of *Aedes aegypti* is successful, due to reduced competition for resources, such as breeding sites. This risk increases with larger scale, longer-term experiments. Brazilian experts have warned that dengue may mutate so that *Aedes albopictus* becomes a more important dengue vector in such circumstances.[61] The potentially devastating effect of a single mutation in the virus has already been observed with chikungunya.[62]

In the case of zika, some scientists have argued that *Culex* species of mosquitoes, which are common in the Cayman Islands and elsewhere, may also play an important role in transmission of disease.[6364,65] Although the evidence is not definitive (and some scientists have found that *Culex* species do not appear to transmit zika in some regions[66,67,68]) at a meeting in Florida in October 2016, three independent groups from Canada, China, and

Brazil presented and discussed laboratory and field data strongly suggesting that the southern house mosquito, *Culex quinquefasciatus*, also known as the common mosquito, is highly likely to be a vector for zika in certain environments.[69] If this is the case, attempting to reduce zika transmission by targeting *Aedes aegypti* may be the wrong approach.

## 2. Costs and cost-effectiveness

The Cayman Islands' 2016/17 budget does not make clear how the proposed releases will be funded. The total budget for mosquito control is projected to increase from $5,797,106 in 2015/16 to $9,609,900 for the 18 month period from 1 July 2016 to the end of 2017 (of which $6,406,600 is to be spent in 2017)[70]. There is a breakdown of this figure in the Annual budget statements, covering seven areas (larviciding; adulticiding; the MRCU's call-out service; scientific advice; the education programme; non-chemical control, such as draining swamps; and disease prevention and control, including surveys).[71] Although this document includes a commitment to "*Reduce the abundance of Aedes aegypti mosquitoes by implementing a sterile male release program based on the Oxitec RIDL genetically modified mosquito*" (page 165), the budget breakdown does not appear to include any costs for the production or release of Oxitec's GM mosquitoes.

In 2011, Oxitec published a paper claiming that its technology is cost effective at preventing dengue.[72] The computer modelling in this paper was conducted before any experimental results were published and is therefore out of date. The numbers of GM mosquitoes that would be needed to prevent a single case of disease remains speculative due to the problems discussed above, and (at best) appears to be much higher than Oxitec originally supposed, even if disease prevention is achievable (which is still unknown). This paper also contains some estimates of costs, based on the costs of constructing and operating facilities to produce sterile insects using irradiation (the sterile insect technique, SIT). Costs are given in US dollars at 2008 prices. The cost of construction of a SIT facility varies considerably from $50,000 to $26 million. The cost of production of sterile insects is given as a mean of $813 per million insects (range $172 to $1639 per million insects). In Brazil, cost of production of irradiated SIT Medflies ranges from $400 to $700 for every million flies released and experts question whether Oxitec's GM flies could be produced any cheaper.[73] Comparable costs are not available for mosquitoes. However, Oxitec's new GM mosquito factory in Piracicaba, which commenced construction in June 2016, is expected to cost £2.5m to £3m according to the company's accounts.[74] The factory aims to produce 60 million GM mosquitoes per week.[75] However, prices are not given and Oxitec's 2015 accounts state: "*It may be some time before the Company's investments in Brazil lead to a self-sustaining cash flow*".

The cost of production of Oxitec's GM mosquitoes may not be directly comparable to the production of irradiated insects for use in SIT programmes. A few press reports have provided financial figures relating to GM mosquito production. In October 2016, Science reported: "*Oxitec's mosquitoes are currently more expensive. The Piracicaba expansion will cost the city roughly $1.1 million over 2 years—some $10 per person in the treated area—about half of which will come out of the existing mosquito control budget. Oxitec itself is paying even more than that, says Slade, but it's too early to tell how much the mosquitoes will cost if they are reared on a much larger scale. "It's only when you roll up your sleeves and build a factory that you know what your costs are.'*". If the cost is the same per person for Grand Cayman (population of 52,601) as the (subsidised) cost is in Piracicaba, this would amount to $526,010 for experimental control of *Aedes aegypti* over a two year period: however, it is important to note this is not the full cost of production (which has not been reported), nor is it known whether the expansion is sufficient to prevent disease. In 2014, the release of 300,000 GM mosquitoes in Panama was reported to have cost $620,000 (more

than $2 per mosquito).[76] At this price, releasing 7 million GM mosquitoes a week (if, as predicted, this is necessary to suppress a wild population of 20,000 mosquitos) would cost considerably more: $14.5 million a week. Alternatively, if we use the fact that Oxitec released 14,000 GM mosquitoes per hectare per week towards the end of its first Grand Cayman experiments, this would suggest that 273 million GM mosquitoes per week might be needed to cover the whole island (19,500 ha). At the reported Panama prices, this would cost more than $564 million a week; at a cost of $500 per million mosquitoes (comparable to SIT), it would cost $136,500 a week (more than $7 million a year if the releases were ongoing and could not be reduced). Any of these options would be likely to be prohibitively expensive, but much more clarity on costs is needed, due to the large variation in the estimates of both numbers needed to be released and production costs, and the absence of any confirmed published figures.

There is an important distinction to be made between Oxitec's proposed commercial service and alternative approaches to vector control, which are normally developed and conducted on a non-commercial basis.

Any costs would be an addition to the mosquito control budget, as existing control measures, many of which focus on controlling other species, would also need to be maintained. Further, it is currently unknown whether releasing even very large numbers of GM mosquitoes would be sufficient to have an impact on the relevant diseases. Even if GM mosquitoes can reduce disease, it is hard to estimate the numbers of GM mosquitoes that would be needed, based on the results of Oxitec's experiments discussed above. If population suppression did occur and had a positive effect, releases would need to continue indefinitely as cessation might lead to a rebound in the cases of disease (assuming the releases remained effective over time). This is the basis of Oxitec's business model, which requires a subscription to an ongoing service. It is also unknown whether the numbers released could be reduced over time, or whether they might need to be increased (e.g. due to resistance developing), or supplemented with other measures (due to other species such as *Aedes albopictus* becoming more of a problem). In fact, in its application to release GM *Aedes aegypti* mosquitoes in the Cayman Islands, Oxitec assumes that future releases of GM *Aedes albopictus* will also be needed.[77] Oxitec's GM *Aedes albopictus* have never been tested in open air releases, so it is unclear whether this approach would work, or how much it would cost.

Since the health of members of the public should not be put at risk, clear protocols are needed to show how GM mosquito releases can be combined with conventional vector control measures which tackle all relevant species. This will add to costs, as measures such as the use of larvicides, adulticides and removal of breeding sites will harm or destroy released GM mosquitoes as well as the wild population.

### 3. Are the Cayman Islands' regulations adequate?

"*The critical path for GMM [Genetically Modified Mosquito] development will include not only proof of efficacy, but also proof of acceptability and deliverability. Risk analysis, community and other stakeholder engagement, and regulatory approval all contribute to proof of acceptability. Cost-effectiveness of the technology vs. other available disease control methods also may influence acceptability. Deliverability will require consideration of an operating model with appropriate prospects for financing to support deployment and subsequent monitoring, sufficient technical and production capacity, quality control processes, methods for management and mitigation in the case of adverse effects, as well as commitment to ongoing stakeholder engagement*". World Health Organisation, June 2014.[78]

At the time of the first experimental releases of Oxitec's GM mosquitoes in the Cayman Islands in 2009-2010, the company was criticised because no biosafety law had been implemented and no risk assessment had been published or been subject to a public consultation.[79,80] People were not properly informed that the mosquitoes were genetically modified and were wrongly told that they were sterile.

Subsequently, the National Conservation Council (NCC) was established under the National Conservation Law 2013[81], which states that its Director may "*develop criteria for determining whether wild populations or proposed introductions of alien or genetically altered species might cause harm to any of the natural resources of the Islands and procedures for regulating and controlling such populations and introductions*" (Article 6(2)(k)). Article 35 requires anyone who wishes to introduce or release in any part of the Islands a live or viable specimen of an alien or genetically altered species to apply to the Council under this Law for a permit to do so. The Chief Agricultural and Veterinary Officer must also consult the NCC when considering whether to permit and import of a genetically altered species.

In 2016, new experimental releases were the subject of a legal challenge through judicial review. The judge found that the NCC and the Department of Environment considered the potential risks before granting approval, and found that there was not a failure to consult with the public before the council's decision.[82,83] However, the judge also advised that the Department of Environment and the NCC begin to develop the "*criteria, procedures and subsidiary legislation*" for determining whether the introduction of alien or genetically modified species might cause any harm to natural resources and for regulating and controlling such populations and introductions.

In October 2016, the Cayman Compass reported that the NCC had prepared a draft policy covering requirements for environmental impact assessments for genetically modified organisms.[84] However, the draft policy has not been published or put out for public consultation. This report also stated that the Mosquito Research and Control Unit (MRCU) had been granted permission to vary the terms of its permit, to allow it to transfer Oxitec's GM mosquito pupae within sealed devices to a new insectary within the grounds of the MRCU prior to release, in order to increase the production and release rate during wet season. The permit was also altered to allow for an additional kilogram of eggs to be imported, increasing the total from 1.65 to 2.65kg, although the restriction to release 22 million mosquitoes remains part of the permit conditions.

In evidence to the court, the relevant authorities relied heavily on the existence of an environmental risk assessment issued by the US Food and Drug Administration (FDA) for proposed releases of Oxitec's GM mosquitoes in the Florida Keys. However, this FDA authorisation has since been withdrawn.[85,86] Further, the risk assessment applied to a small initial trial in different habitat, and the USA has no specific guidance on risk assessments for GM mosquitoes and is not a member of the Convention on Biological Diversity or the Cartagena Protocol on Biosafety (CPB).

The National Conservation Law 2013 gives effect to a number of conventions, including the Global Convention on Biological Diversity (CBD). The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity is an international agreement which aims to ensure the safe handling, transport and use of genetically modified organisms (GMOs), known as living modified organisms (LMOs) under the Protocol.

Developing the necessary criteria, procedures and subsidiary legislation to properly assess the risks of scaled up releases, and regulate the use of this technology, is not a trivial task.

Guidance published by the European Food Safety Authority (EFSA) outlines the evidence that Oxitec would need to provide for its GM mosquitoes to be placed on the EU market (placing on the market means making available to third parties, whether in return for payment or free of charge).[87] Pages 73 to 107 of the EFSA Guidance provide details on specific areas of risk for GM insects. Under the CPB, the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management has also produced Guidance on the Risk Assessment of Genetically Modified Mosquitoes.[88] In addition, the recommendations of the WHO's Vector Control Advisory Group (VCAG) will need to be implemented, including the need for proper scientific protocols to assess the impacts of the proposed releases on all relevant diseases and the need for rigorous independent monitoring and evaluation.

The risks of any trial increase as the numbers are scaled up. Issues include:[89,90]

- The survival and spread of GM mosquitoes, including biting females, and their impacts on the environment and human health. Scaling up releases means that greater numbers will survive to adulthood, and these numbers may increase if the GM mosquitoes encounter sufficiently high levels of tetracycline in the environment to deactivate the genetic killing mechanism, or if they evolve resistance to it.
- Impacts of the use of a non-native strain of mosquito, which may introduce new traits into the wild mosquito population, including different disease-transmission properties.
- Disposal of the antibiotic tetracycline used to breed the GM mosquitoes in the lab, and the issue of whether released GM mosquitoes will spread antibiotic resistant bacteria into the environment.
- Impacts on other species, including the question of whether population suppression of *Aedes aegypti* mosquitoes could lead to an increase in numbers of *Aedes albopictus* mosquitoes, or other disease-transmitting species.
- Questions regarding the impact of the releases on the spread of tropical diseases, including efficacy and risks (including impacts on immunity).

Larger scale releases also increase the risk of problems such as the release of biting females or GM males which have evolved resistance to the killing mechanism and can breed successfully; or the release of mosquitoes which have been contaminated with infectious disease e.g. if they have been accidentally fed with infected animal blood, or if wild infected mosquitoes have somehow entered the insectary. Oxitec currently feeds its GM insects on horse blood from the UK, which will not be infected with tropical diseases which are not present in that country. However, scaling up production of GM mosquitoes in the Cayman Islands may mean a new supply of blood for feeding will be needed, and testing for infection will become essential.

It is widely recognised that fully informed consent from the public is needed for releases of genetically modified mosquitoes.[91,92] Fully informed consent requires an opportunity for prior consultation on the risk assessment, as the public must be properly informed about the risks.

Fully informed consent to medical research is a requirement of the World Medical Association's Helsinki Declaration (which covers the ethical responsibilities of medical professionals).[93] For example, all medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation (Article 17); the design and performance of each research study involving human subjects must be clearly described and justified in a research protocol (Article 22); the study must be approved by an ethics committee (Article 23) and participants must be fully informed about the study, including potential risks (Article 26). There must therefore be public recognition that the releases are

still experimental, and are not a proven approach to tackling the risk of zika, dengue or chikungunya.

### 4. Have alternatives been properly considered?

"*While we acknowledge that to hold novel or experimental techniques to unrealistically high standards is counterproductive, it is surely uncontroversial to suggest that diverse and credible data must be publicly available before resources and attention are diverted away from current control programmes. Furthermore, in the specific context of ongoing mosquito control it is essential that proponents of any new approaches (biotechnological or otherwise) make efforts not to undermine confidence in techniques likely to remain part of frontline responses*". [94] Boëte & Reeves, 2016.

The World Health Organisation's Strategic Response Plan for Zika includes an objective to prevent adverse health outcomes associated with Zika virus infection through integrated vector management, risk communication and community engagement.[95] The approach includes:

● implementing integrated vector management (IVM) to efficiently and judiciously use resources, defined as "*a rational decision-making process for the optimal use of resources for vector control*";
● targeting all life stages of the *Aedes* mosquito: egg, larva/pupa and the adult;
● reducing the risk of sexual transmission and other possible routes of transmission;
● coordinating, collaborating and partnering with stakeholders from government (municipalities, ministries of education, health, social services, water and sanitation, etc.) and civil society (NGOs, private sector, faith-based associations, churches, etc.);
● engaging and empowering communities, private sectors, etc. in mosquito control and prevention behaviours at the environmental, household, schools, businesses, personal levels, etc.; and
● developing relevant risk communication and behaviour change strategies and materials.

The WHO zika strategy also aims to fast track and scale up the research, development and availability of *Aedes* mosquito control tools, diagnostic tests and vaccines.

For dengue, the WHO notes that one vaccine has already been licensed, and five more are under development.[96] Vector control measures include:

- preventing mosquitoes from accessing egg-laying habitats by environmental management and modification;
- disposing of solid waste properly and removing artificial man-made habitats;
- covering, emptying and cleaning of domestic water storage containers on a weekly basis;
- applying appropriate insecticides to water storage outdoor containers;
- using of personal household protection such as window screens, long-sleeved clothes, insecticide treated materials, coils and vaporizers;
- improving community participation and mobilization for sustained vector control;
- applying insecticides as space spraying during outbreaks as one of the emergency vector-control measures.

The WHO states that active monitoring and surveillance of vectors should be carried out to determine effectiveness of control interventions.

Any decision about future use of Oxitec's GM mosquitoes must consider all the alternatives and whether the money could be better spent.

### Conclusions and recommendations

Before any further open releases of GM mosquitoes are considered:

- Decision-makers in the Cayman Islands must thoroughly consider whether releases of Oxitec's GM mosquitoes are effective; how much they cost and whether they are cost-effective; and the existence of alternatives. The relevant information, including the results of Oxitec's 2016 trials and estimates of future pricing, should be made publicly available.
- The National Conservation Council (NCC) and Department of Environment must develop the necessary criteria, procedures and subsidiary legislation to properly assess the risks of scaled up releases, and regulate the use of this technology. Policies should be subject to public consultation;
- The recommendations of the WHO's Vector Control Advisory Group (VCAG) should be implemented, including: the need for proper scientific protocols to assess the impacts of the proposed releases on all relevant diseases; and the need for rigorous independent monitoring and evaluation;
- Much more openness is needed about the proposed trials, including detailed answers to specific questions.

In particular, a clear process needs to be set out for decision-making on proposed future trials. Some specific questions that need to be answered are:

- What are the scientific protocols for the proposed future trials, including: epidemiological and population endpoints to measure impact on adult female mosquito populations and risk of disease (for zika, dengue and chikungunya); and the protocols for combining GM mosquito releases with other vector control measures, including spraying?
- How will independent evaluation and monitoring of the proposed trials be implemented? How will monitoring be funded?
- What is the proposed procedure for environmental risk assessment and will the draft policy and regulations be published and be subject to public consultation?
- Once the procedure is established, will future environmental risk assessments be subject to public consultation?
- What will the procedure be for amending or expanding licences?
- What is the purpose of the amendment to the existing permit reported in October 2016, which allows GM pupae to be transferred to a new insectary? Does this mean there have been breeding problems, and what are the implications for the proposed experiments?
- What are the expected future costs of the trials and future annual costs, and how is this intended to be funded? As well as the production costs of the GM mosquitoes, what are the costs of sufficient technical and production capacity, quality control processes, methods for management and mitigation in the case of adverse effects, and ongoing stakeholder engagement?
- Will the results of the current trials be published in a peer reviewed journal before a decision to expand the trials is taken?
- Will there be any independent scrutiny of Oxitec's trial results to date?
- What evidence currently exists regarding the presence and role of *Aedes aegypti* and other mosquito species in transmitting zika, dengue and chikungunya on the Cayman Islands? For example, will monitoring trap data and mosquito population estimates be published? Will locations of cases of these diseases be published, including the locations of the non-published zika cases of 2016, as well as evidence regarding disease transmission for all species (including *Aedes albopictus* and *Culex* species)?
- What measures will be taken to assess and address potential impacts of GM mosquito trials on other mosquito species? Will a proper study be undertaken of the

potential impacts of other species on disease, prior to any decisions on undertaking further trials?

- How will other potential risks be more thoroughly assessed? For example: will feeding trials be used to test the risk of consumption of GM mosquitoes by other species; will the presence of antibiotic resistant bacteria be investigated before GM mosquitoes are released; will potential breeding sites, including septic tanks, be surveyed for tetracycline contamination; will the GM mosquitoes be tested for disease transmission properties before they are released?
- What quantities of the antibiotic tetracycline will be used during the GM mosquito breeding process, how will this be regulated, and how will disposal be controlled and monitored?
- If new releases do take place, how will impacts on other mosquito species be monitored, in the short- and long-term?
- Will protocols be developed and published to minimise and detect the risk of the accidental release of biting female mosquitoes; the use of contaminated feed; and the evolution or accidental introduction of GM mosquitoes which are resistant to the killing mechanism?
- Will an economic report be published prior to any decision, assessing the potential effects of long-term wide-scale releases of GM mosquitoes on the tourism industry?

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