Oxitec's Genetically Modified Mosquitoes: Ongoing Concerns

GeneWatch UK

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UK biotech company Oxitec has released millions of genetically modified (GM) mosquitoes in Brazil, following smaller experiments in the Cayman Islands and Malaysia. Further experiments are planned in Brazil on an even larger scale. The company also hopes to conduct experimental releases in Panama, the USA (Florida Keys), India, Sri Lanka and perhaps other countries.

Oxitec's patented technique for genetically modifying insects is known as RIDL (Release of Insects carrying a Dominant Lethal genetic system).[1] 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.

Oxitec's male OX513A GM mosquitoes are intended to mate with wild females and produce offspring which die as larvae. Releases of large numbers 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 Yellow Fever mosquitoes (*Aedes aegypti*) which transmit the tropical disease dengue fever.

No risk assessment was published for public scrutiny or consultation prior to releases of GM mosquitoes in the Cayman Islands or Brazil. In Malaysia only a summary was published. GeneWatch has obtained copies of the risk assessments in the UK because they must be provided by Oxitec when exporting GM mosquito eggs for open release for the first time to a given country. In no case did the company correctly follow this notification procedure, with the result that there was no independent scrutiny of whether these risk assessments met the required European standards.

There remain many concerns about Oxitec's technology. Some unanswered questions are highlighted below.

Issues include:

- The results of Oxitec's population suppression experiments in Cayman and Brazil have not been published in scientific journals, but information in the public domain suggests that RIDL may not be particularly effective at suppressing mosquito populations and could even be less, not more, effective than the Sterile Insect Technique (SIT) using irradiated insects.
- Ineffectiveness is a matter of particular concern in dengue endemic areas because in some situations partial or temporary suppression of mosquito populations could make the dengue situation worse.
- Oxitec did not correctly follow the procedure for transboundary notification of shipments of GM mosquito eggs overseas: the practical consequence of this is that risk assessments were not made publicly available prior to open release trials and did not meet the necessary standards.
- Numerous important issues were therefore not properly considered before millions of GM mosquitoes were released in to the environment in the Cayman Islands and

Brazil. Smaller experiments in Malaysia did include a consultation process, however there were some deficiencies with the process which need to be addressed.

- In its publicity about the trials, Oxitec has oversimplified the complex relationship between *Aedes aegypti* mosquitoes, other mosquito species, the humans that are bitten, and the four serotypes of dengue virus. This means that most potential adverse impacts have effectively been excluded from public debate, the risk assessment process, and the process of seeking consent from local populations.
- Oxitec has repeatedly referred to its GM mosquitoes as sterile, when this so-called sterility is partial and conditional. The GM mosquitoes do breed and most die at the larval stage: the extent to which their offspring survive to adulthood is one of many factors which influences the efficacy and safety of this approach.
- The decision to scale-up experiments in Brazil appears to be driven by a political agreement to commercialise Oxitec's technology there, rather than by a thorough assessment of the likely risks and benefits.

Will releasing GM mosquitoes suppress wild mosquito populations?

Oxitec frequently compares its RIDL technology to the Sterile Insect Technique (SIT). SIT involves releasing large numbers of irradiated insects to mate with wild ones. Since the irradiated insects are sterile, no offspring are produced and this can be effective in reducing insect populations. Chemical methods of sterilisation have also been tested. SIT has been used successfully with some agricultural pest species, but has been less successful with others because different insect species have very different life histories and behaviours.[2] In general SIT is not effective at reducing high density populations of insects without first using other conventional approaches to reduce the population, but it may be effective at reducing or eradicating smaller, isolated populations.[3] However, SIT has not generally been successful for mosquitoes, where population suppression has been achieved only in a few experiments with very large "release ratios" of sterile to wild mosquitoes.[4,5] Although there have been a number of field trials of mating fitness and other factors, only two population suppression trials have been conducted using SIT for *Aedes aegypti* mosquitoes. These took place during the late 1970s in an isolated Kenyan village, using semi-sterile males which had a fertility of 37%. In the first experiment, the estimated release ratio of about ten to one (released to wild males) had only a small effect on population levels and was ineffective compared to removing larvae from domestic water containers in a neighbouring village.[6] A second SIT experiment was conducted a little later in the same locality with similar results.[7] Two genetic markers that were carried by released males but unknown to the region persisted in the population during the entire period of observation of nearly a year after the experiments.[8]

SIT is not currently in use for any species of mosquito, however there are current research programmes investigating the potential use of SIT using irradiated *Anopheles arabiensis* mosquitoes (which transmit malaria) in Sudan[9]; and irradiated *Aedes albopictus* mosquitoes (which transmit dengue and other viruses) in Italy.[10] Recent experiments with *Aedes albopictus* suggest that it is possible to adjust radiation doses so that these mosquitoes are sterilised without significant loss of mating fitness.[11]

Applying SIT to mosquitoes is complicated by what scientists call "density dependent" effects on mosquito populations.[12,13] The size of a population of mosquitoes does not depend only on how well the mosquitoes reproduce but also on other factors such as competition for food between larvae and for breeding sites. Reducing reproductive fitness may have little effect if the size of the mosquito population is limited mainly by these factors, rather than by its ability to reproduce.[14] Density dependent effects mean that reducing the numbers of mosquitoes that breed successfully can sometimes have little effect on total numbers of adult mosquitoes and paradoxically might sometimes even increase populations: for example, because reducing breeding success also reduces competition between larvae for resources,

resulting in increased survival rates or a rebound in numbers. Density dependent effects can influence the current generation of mosquitoes or only affect future generations (delayed density-dependent effects).[15] Density dependent effects tend to be less important for agricultural pests in large-scale monoculture agriculture, because their food supplies are effectively unlimited, so competition for resources can play a less important role.

Influx of mosquitoes from neighbouring areas into an area where the population is suppressed can be a major problem with the use of SIT. Incomplete sterilization, reduced mating competitiveness (compared to wild mosquitoes) and immigration of mosquitoes from surrounding areas can all reduce the effectiveness of SIT.[16] *Aedes aegypti* eggs can survive several months under dry conditions in a dormancy state at the end of their embryonic development and this adds to the difficulties of controlling populations, which can spread through dispersal of the eggs.[17] The US Centers for Disease Control and Prevention (CDC) suggests that even if all larvae, pupae, and adult *Aedes aegypti* were to be eliminated at once from a site, its population could recover two weeks later as a result of egg hatching following rainfall or the addition of water to containers harbouring eggs.[18]

Oxitec argues that RIDL will be more successful for mosquitoes than SIT for two main reasons: (1) RIDL males will be fitter than irradiated ones and will compete more successfully with wild males to mate with wild females; and (2) because the offspring of the GM mosquitoes survive to the late-larval or early-pupal stage, they will also compete with wild larvae for food, further supressing the wild mosquito population. Oxitec makes these predictions based on a number of computer models which aim to forecast how mosquito populations will respond to large-scale releases of its GM mosquitoes.[19,20,21,22] These models all contain many simplifying assumptions, including assumptions about how density dependence affects the population of mosquitoes. These computer models build on a model built using data on the development of adult and larvae *Aedes aegypti* mosquitos in Thailand[23]: they have not been validated with data from the areas where Oxitec has conducted its releases. The model results indicate that the dynamics of mosquito populations are not straightforward and that releases of sterile or RIDL mosquitoes can have counter-intuitive effects, including oscillations in adult abundance that may be above usual population levels and increases in mosquito populations in surrounding areas. If such increases in mosquito populations at or near the release site occur, they might pose a risk to human populations: Oxitec predicts that these risks occur with SIT but not with RIDL. It is unclear how well these computer models represent what would actually happen in the real world: it is therefore possible that neither SIT nor RIDL poses these risks, or that both approaches do. There is no evidence in practice that competition between RIDL larvae and wild larvae enhances the effectiveness of SIT. An added complication is that the effects of larval interactions on mosquito populations are different in different contexts, because they may be altered by ecological conditions.[24]

In experiments in the Cayman Islands in 2010, Oxitec measured the effect of releasing about 3 million GM OX513A mosquitoes on local populations. Oxitec's claim that an 80% reduction in the *Aedes aegypti* population was achieved has been widely reported in the press and some data have been shown in company presentations.[25] However, only the results of a small pre-trial release of GM mosquitoes in Cayman have been published in a scientific journal: this smaller trial did not involve an attempt to suppress the wild population.[26,27] In January 2011, Oxitec submitted the results of its Cayman Islands population suppression trial to the journal Science[28], but no publication has yet appeared. Importantly, this means that the "release ratio" (of GM to wild mosquitoes) has not been published and the details of the experiment have not yet been subjected to independent public or scientific scrutiny.

The paper may have been rejected or delayed by Science for ethical or scientific reasons. It is unclear whether Oxitec did sufficient research to establish the baseline levels and

fluctuations in the wild population of mosquitoes before it conducted its experiments: there were no existing data on *Aedes aegypti* presence at the chosen sites because Oxitec sought areas with no mosquito control measures that had not previously been monitored. [29] The company claims to have measured an 80% difference between numbers of mosquitoes in the release area and the neighbouring area, but this leaves unanswered the potential concern that the releases might lead to increases of mosquitoes in surrounding areas. In presentations, Oxitec has also suggested that it had to significantly increase its releases of GM mosquitoes, beyond the initial expected levels, in order to achieve the observed population suppression effect. If the reported observed effect required a very high ratio of GM mosquitoes to wild ones this would suggest that the technology was relatively ineffective and failed to overcome the problems observed in past attempts to use SIT for mosquitoes. Press reports have suggested that high release ratios of ten to one GM mosquitoes to wild mosquitoes may be required.[30]

Preliminary results from the experiments in Brazil show that a release ratio of fifty-four RIDL to one wild type male was used in the final phase of the experiments conducted in Brazil. The mating competitiveness was only 0.03 (3 in 100) on average and dropped to 0.012 (1.2 in 100) in the final phase.[31] The number of mosquitoes trapped in the untreated area also increased in the final phase of the experiment. More than half a million mosquitoes a week were produced during this late phase of the experiments and the releases were concentrated in a small area of houses in Itaberaba (Bahia), less than 500m by 200m. The high release ratio and low mating competitiveness suggest that RIDL may in fact compare poorly with SIT using irradiated insects, rather than being significantly better. Prior to conducting Oxitec's experiments in Brazil experiments, lead researcher Prof Margareth Capurro predicted that a release ratio of five to ten GM mosquitoes to wild mosquitoes would be needed.[32]

The results of Oxitec's population suppression experiments in Cayman and its experiments in Malaysia and Brazil have not been published in scientific journals, but information in the public domain suggests that RIDL may not be particularly effective at suppressing mosquito populations and could even be less, not more, effective than SIT using irradiated insects. Ineffectiveness is a matter of concern if this means more effective approaches are neglected. More seriously, in some circumstances partial or temporary suppression of mosquito populations could make the dengue situation worse, as discussed below. Further, even if RIDL is temporarily successful at reducing the *Aedes aeygpti* mosquito population, this might not have the desired effect on dengue fever, and the effect might not be sustained. These issues are considered in more depth below.

Will population suppression reduce dengue fever?

Although it seems logical to assume that reducing the population of dengue-transmitting mosquitoes will reduce the incidence of the disease, in reality the situation is more complex. Dengue transmission can sometimes continue even with very small numbers of mosquitoes and disease transmission thresholds are unknown.[33,34] In Brazil, one study found the number of dengue cases in two different areas was associated with human population size, and not with the number of mosquitoes.[35] Although rainfall (associated with increases in the number of mosquito larvae) plays a role, population density and poverty are both important factors in the incidence of dengue and associated deaths.[36] Local patterns of transmission are complex and the density of the human host population plays a fundamental role in determining the transmission dynamics of endemic dengue.[37]

Dengue fever has a complex immunology, with antibodies against one of the four serotypes sometimes protecting and sometimes enhancing disease severity following infection with a second serotype. The cyclical occurrence of epidemics is likely to be due to an interaction

between the availability of susceptible hosts (e.g., children born after an epidemic), successive waves of different dengue virus strains, and climatic factors.[38]

The most serious and often fatal form of dengue, dengue hemorrhagic fever (DHF), appears to be more likely when a person is infected by a second serotype of dengue fever, having already been infected by one of the other serotypes. This is thought to be due to immunological mechanisms including antibody dependent enhancement (ADE), in which the antibodies developed against the first infection make the second infection more severe. However, if the two infections with different serotypes occur in quick succession (within weeks) cross-immunity can develop which has the opposite effect, reducing the risk of DHF. Many of the individuals in areas of high vector mosquito abundance would be infected by, and acquire immunity against, multiple serotypes while they are protected by this cross-immunity and develop resistance to DHF unknowingly. One concern about partially effective interventions to reduce mosquito numbers is that as the mosquito abundance decreases, an increasing number of individuals would experience secondary infections after the protective cross-immunity has waned, and the incidence of DHF would then increase. One study in Thailand has suggested that in regions of intense transmission, insufficient reduction of mosquito populations may increase long-term incidence of DHF, because of the existence of this complex cross-immunity effect.[39,40] This analysis suggested that reducing *Aedes aegypti* abundance from the highest level in Thailand to a moderate level would increase the incidence of DHF by more than 40%. Further computer modelling of this data has confirmed this finding.[41] If correct, this has major implications for dengue control programmes, including the use of Oxitec's GM mosquitoes. It suggests that ineffective programmes may be worse than useless because they can actually increase the harm due to the disease, at least in high risk areas.

Only one serotype of the dengue virus was originally included in Oxitec's computer models, so possible adverse effects due to interactions between the four different types of virus and human immunity were not explored.[42] A more recent model includes two serotypes but does not include the short-term benefits of cross-immunity and therefore cannot predict the problem identified in Thailand: Oxitec's model assumes that reducing mosquito numbers can only lead to benefits.[43]

Several authors have warned that an initial reduction in mosquito numbers could lead to a reduction in human immunity in dengue endemic areas. This effect on immunity, combined with residual disease transmission (from *Aedes aegypti* or *Aedes albopictus*), could result in a "rebound" effect, in which the amount of serious disease increases, despite a reduction in the numbers of *Aedes aegypti* mosquitoes.[44,45] Two of Oxitec's shareholders are co-authors on a paper which studied the rebound effect in Singapore.[46] Although in this case the rebound occurred with traditional public health approaches, it is possible that a rebound could be more serious if a technology is used that becomes less effective over time (with effects similar to implementing and then stopping a public health programme to remove breeding sites). This means that concerns that resistance to RIDL may develop (see below) need to be considered very seriously, because using a technology that is only temporarily effective might also make the dengue situation worse.

Concerns about human immunity are applicable to areas where dengue is endemic, such as in Brazil, Panama or Malaysia. However, Oxitec has also tested or proposed testing its technology in sites such as the Cayman Islands and the Florida Keys, where only sporadic cases of dengue occur. Cases in these areas often originate from a single person infected while they were abroad, who may then be bitten by a mosquito that then transmits disease to others in the area. Because only a small number of mosquitoes are needed for disease transmission, it is possible that reducing mosquito populations has only a marginal effect on the incidence of dengue in these circumstances.

Changes in population structure (for example, mosquito size), as well as abundance, might also alter dengue transmission.[47] A further possibility that has not been well explored is that the dengue virus could evolve to become more virulent: this is considered to be a lower risk with population suppression approaches than with attempts to modify disease transmission, but very little research has been conducted.[48]

Recently, a group of researchers have discussed the difficulties in assessing whether GM mosquito releases will have a beneficial impact on the incidence of dengue and proposed monitoring potential impacts on disease by measuring the antibodies people develop against the virus.[49] However, Oxitec continues to make unsubstantiated claims that a reduction in the population of *Aedes aegypti* mosquitoes will lead inevitably to a reduction in the incidence of dengue.

Low disease transmission thresholds mean that even if Oxitec's technology were successful in suppressing wild mosquito populations it might not be of benefit in reducing dengue fever. In some situations in endemic areas, a rebound effect might even make the problem worse, or the incidence of the more serious fatal form of the disease, dengue hemorrhagic fever, might increase: these problems are more likely to arise if the technology is of temporary or limited effectiveness at suppressing the population of *Aedes aegypti* mosquitoes. Oxitec has released large numbers of GM mosquitoes in dengue endemic areas of Brazil without any monitoring of the effects on health.

Impacts of the antibiotic tetracycline

Oxitec's OX513A GM mosquitoes have been engineered to be able to survive to adulthood only in the presence of tetracycline (an antibiotic widely used in industrial agriculture and medicine). Tetracycline acts as a chemical switch: in its presence the mosquitoes can be bred for many generations, but in its absence many of them die. The GM mosquitoes are bred to adulthood in the lab in the presence of the antibiotic and then sorted so that mostly males are then released into the environment. The GM male mosquitoes are supposed to mate with wild females and their offspring are then supposed to die in the absence of tetracycline, mostly at the larval stage.

Oxitec's 'conditional lethality trait' is created by genetically engineering the mosquitoes to express a protein called tTa (tetracycline-controlled transactivator). High level expression of tTA is toxic to the mosquitoes and kills them at the larval stage, although the mechanism for this is not fully understood. Tetracycline binds to tTa and prevents it leading to the expression of more tTA, allowing the mosquitoes to survive to adulthood. This allows the mosquitoes to be bred in the laboratory by including tetracycline in their feed.

In the lab, Oxitec found that the offspring of its GM mosquitoes had a 3-4% survival rate even in the absence of tetracycline.[50] However, the survival rate could be much higher if there is tetracycline contamination in the environment.[51] A confidential Oxitec document, made public by NGOs, reveals that the offspring of GM mosquitoes fed on cat food had a 15% survival rate.[52] The cat food was made of industrially-farmed chicken, which was presumed to be contaminated with tetracycline, which is widely used in agriculture: heat-treatment of the chicken when making the cat food was presumed not to have removed all the tetracycline. Oxitec attempted to withhold the information in this document by arguing it was commercially confidential.[53,54]

Large quantities of antibiotics are released daily into the natural environment in effluent and through use in industrialised farming and aquaculture, and the tetracycline class of antibiotics is one of the most commonly used in human and veterinary medicine.[55] This class of antibiotics is detectable in foodstuffs such as meats[56], milk[57], farmed fish[58] and honey[59]; in

animal slurry[60]; and in human sewage[61]. The length of time these antibiotics take to degrade depends on the foodstuff or environmental medium and other factors such as temperature.

Oxitec and the research partner that fed the GM mosquitoes on cat food have claimed that tetracycline is not present in the environment in sufficient quantities to ensure survival of the GM mosquitoes and that the *Aedes aegypti* species is not found in any sites liable to pollution by tetracycline from any source.[62,63,64] To reach this conclusion, Oxitec has reviewed the literature on tetracycline contamination in municipal sewage plants, but has not considered any other source of tetracycline or other antibiotics in the same family of chemicals. Human and animal sewage is often contaminated with tetracycline and may be present in domestic sewage systems including cesspits, septic tanks and animal manure and slurry. Industrially-farmed chicken is clearly present in the environment, in cat food and elsewhere e.g. discarded take-aways. The levels of tetracycline or a related chemical must have been high enough and persistent enough in the cat food to give the reported survival rate, but Oxitec does not appear to have identified the cause or shown that similar levels do not occur in other food or feed products or elsewhere. Nor has Oxitec established a threshold which excludes the possibility that lower levels of tetracycline or related chemicals could also cause survival problems, or published any information on the dose-response curve.

Third World Network (TWN) has published a briefing paper which cites extensive evidence that *Aedes aegypti* mosquitoes do breed in sewage-contaminated water, where tetracycline may be present.[65] Although *Aedes aegypti* usually breed in small pools of water, such as water butts and flower pots, they have been found in septic tanks in Nigeria, Puerto Rico and Florida and several studies have confirmed that they can breed in sewage-contaminated water.[66,67,68,69,70] The authors of the Puerto Rico study calculated that septic tanks they studied could produce more than 18,000 *Aedes aegypti* adults per day and concluded that breeding in septic tanks could contribute significantly to the maintenance of the dengue virus on the island.[71] In 2004, there were more than 36,000 septic systems and 5,000 to 10,000 cesspits in the Florida Keys, where Oxitec plans to undertake its next experiments.[72] In Brazil in 2005, 78 million inhabitants had septic tanks.[73] Areas such as city slums that lack piped water (a risk factor for dengue) usually also lack access to centralised sewage systems.[74]

In one US study, concentrations of tetracycline resistance genes in bacteria measured in effluents from modern septic tanks were several orders of magnitude higher than those in treated municipal effluent: however, concentrations of tetracycline in the septic tanks were not reported.[75] Tetracycline concentrations of up to 65.2 µg/L have been found in municipal sewage in one study in China[76], higher than the 10 µg/L level that Oxitec suggests is a "threshold" for increased survival (based on limited, unpublished data[77]). It is unknown whether similar concentrations could occur in some *Aedes aegypti* breeding sites (such as septic tanks, cess pits or areas contaminated with food or farming waste) because the relevant research has not been done.

The reliance of Oxitec's RIDL technology on tetracycline is a fundamental flaw because tetracycline is widely used in medicine and veterinary practice and is often present in sewage, slurry and industrially-farmed animals and foods. Oxitec has provided insufficient information to establish the survival rates of its GM mosquitoes in tetracycline-contaminated environments such as septic tanks. A high survival rate of the offspring of the GM mosquitoes would make Oxitec's technology less effective at suppressing the population of wild mosquitoes and would also mean that large numbers of female GM mosquitoes (which bite and can transmit disease) might survive and breed, perhaps for multiple generations.

Impacts of population changes on other mosquito species

Dengue fever is also transmitted by the Asian Tiger mosquito, *Aedes albopictus*, as are several other viruses.[78] *Aedes aegypti* originates from Africa and spread to many areas in the tropics with the slave trade. *Aedes albopictus* originates from south-east Asia but has dramatically expanded its range to many tropical, subtropical and temperate regions, both in urban and rural areas around the world. Both species can spread extremely rapidly and can interact with and displace one another: for example, *Aedes albopictus* has replaced *Aedes aegypti* in much of Florida and in Bermuda.[79,80]

If releases of GM *Aedes aegypti* mosquitoes are successful in suppressing the wild population of this species, it is possible that numbers of *Aedes albopictus* could increase due to reduced competition for breeding sites and food. This risk is rated 'medium' in the report of the NRE-UNDP-GEF workshop on Risk Assessment of Transgenic Insects in Malaysia in November 2008, as reported in a publication by Oxitec's Regulatory Affairs Manager, Camilla Beech, and others[81]. Most other interventions to reduce mosquito populations (e.g. removal of breeding sites, mosquito traps, larvicides, spraying) are not species specific, so that this issue does not usually arise. However, because RIDL targets a single species at a time, there is a risk that if *Aedes aegypti* numbers are reduced, *Aedes albopictus* could increase in numbers or become established in areas where it was not previously a problem.

Aedes aegypti and *Aedes albopictus* are different species with different behaviours, but the habitats of the two species partly overlap, they can breed in the same sites, and competition between larvae can affect their relative abundance.[82,83,84] *Aedes albopictus* has been replaced by *Aedes aegypti* in most major cities in South East Asia, where *Aedes aegypti* is now considered the main vector of dengue virus:[85] however, both species can still play an important role in disease transmission.[86] *Aedes albopictus* has been responsible for concurrent epidemics of dengue and chikungunya in Gabon,[87] for an outbreak of dengue fever and dengue hemorrhagic fever in Dhaka, Bangladesh[88] and the re-emergence of dengue in southern China.[89] In China, *Aedes aegypti* is generally the vector for dengue in coastal areas, and *Aedes albopictus* is the vector in inland regions.[90] Although different strains exist in different countries and vary in their capacity to transmit disease, *Aedes albopictus* can be a very competent vector of dengue.[91] Its role in disease transmission in South America is poorly known, although this species appears to have played a role in dengue outbreaks in Columbia.[92,93] One study suggests that *Aedes albopictus* is a disturbing threat to dengue control in Brazil and that it could also become the link between the jungle and urban cycles of yellow fever.[94] It appears to be a more invasive species than *Aedes aegypti*[95] and there is some evidence that it may be less susceptible to some insecticides.[96] An increase in *Aedes albopictus* could therefore be harmful to health and its increase or establishment in a new area could be challenging to control.

In some areas of the USA, *Aedes albopictus* has displaced *Aedes aegypti*, but in parts of southern Florida they coexist: a 2004 study found *Aedes albopictus* in the Florida Keys, although *Aedes aegypti* is more prevalent.[97] Sterility caused by cross-mating between *Aedes albopticus* and *Aedes aegypti* (a process known as satyrization) may have initially contributed to the observed competitive reduction of *Aedes aegypti* by invasive *Aedes albopictus* in many areas of Florida.[98]

Competition among larvae may also affect the probability of virus transmission, which may have important consequences for dengue.[99] For *Aedes albopictus*, but not *Aedes aegypti*, competition increases the probability of acquiring disseminated infections of arboviruses. If invasion by *Aedes albopictus* results in competitive replacement of *Aedes aegypti*, so that the two species can coexist, this competitive effect could increase the vectorial capabilities of *Aedes albopictus* compared with that of *Aedes aegypti*. Thus, *Aedes albopictus* may

assume a greater role in dengue transmission, because not only the numbers of *Aedes albopictus* but also its ability to transmit the virus could increase.

Oxitec has published a paper which attempts to model the interactions between *Aedes aegypti* and *Aedes albopictus*, acknowledging that they do compete for resources: this model looks only at the effects on the numbers of mosquitoes, not disease transmission.[100] The model produces many possible outcomes, depending on assumptions. Elsewhere, Oxitec has accepted that it is possible that there could be some increase in the numbers or range of *Aedes albopictus*, but argues that this would "*represent only a marginal reduction in the net beneficial effect of controlling Ae. aegypti*" and that in any case control programmes for both species could be combined.[101] Oxitec has recently published a paper on genetically modified *Aedes albopictus* (a flightless-female version of its technology), although this is at an early stage of development.[102] Its press release emphasises the difficulties of tackling dengue spread by *Aedes albopictus*.[103]

In West Africa and Malaysia the situation may be complicated further by the existence of cycles of dengue transmission involving monkeys and other species of forest-dwelling *Aedes* mosquitoes.[104] Sylvatic (forest) dengue, however, is genetically distinct from urban dengue, and domestic *Aedes aegypti* are not good vectors of the forest form.

There are potential problems with using a species-specific approach to a disease which is transmitted by more than one species of mosquito. If Oxitec is successful at suppressing *Aedes aegypti* mosquito populations this could lead to an increase in *Aedes albopictus*, which is also a vector for dengue and other diseases.

Introduction of new mosquito strains and transmission of other diseases

Different strains of *Aedes aegypti* and *Aedes albopictus* vary in their ability to transmit dengue fever, yellow fever and the chikungunya virus.[105,106,107,108,109] For example, the existence of several apparently distinct lineages of *Aedes aegypti* within Brazil could imply differences in the susceptibility for transmitting dengue and urban yellow fever viruses.[110,111] Introducing new genetically modified strains might alter transmission of one or more of these diseases, especially if the strain released differs from the wild strain present in the area being targeted. For example, the risk of a yellow fever outbreak at some point in the future might be increased by introducing a stain that readily transmits yellow fever into an area where local strains do not. Resistance to insecticides may also vary and introduction of a potentially resistant parent strain must obviously be avoided.[112,113]

There are three types of yellow fever transmission cycle: sylvatic, intermediate and urban. All three cycles exist in Africa, but in South America, only sylvatic and urban yellow fever occur.[114] Sylvatic (or jungle) yellow fever occurs in tropical rainforests where monkeys, infected by sylvatic mosquitoes, pass the virus onto other mosquitoes that feed on them; these mosquitoes in turn bite and infect humans entering the forest, producing sporadic cases. Urban yellow fever results in large explosive epidemics when travellers from rural areas introduce the virus into areas with high human population density. Domestic mosquitoes, most notably *Aedes aegypti*, carry the virus from person to person. These outbreaks tend to spread outwards from one source to cover a wide area. An outbreak of this type occurred in 2008 in Brazil.[115]

Oxitec states that OX513A is available in Asian and Latin American genetic backgrounds[116]. In Cayman, the OX513A insertion in *Aedes aegypti* (originally developed from a Rockefeller strain[117]) was introgressed into a Mexico-derived genetic background by five generations of backcrossing[118]: it appears that this same strain was then used in Brazil. In Malaysia, the parent organism was again the transformed *Aedes aegypti* Rockefeller strain which was subsequently crossed with a more recently acquired Asian strain of *Aedes aegypti* at the

Malaysian Institute for Medical Research (IMR) resulting in the OX513A(My1) strain[119,120]. This strain has been tested for insecticide resistance.[121] The Rockefeller strain of *Aedes aegypti* appears to have been derived from a strain established in Havana, Cuba, by Carlos J. Finlay in 1881, used in the original experiments which established that *Aedes aegypti* mosquitoes are a vector for Yellow Fever.[122,123] Oxitec does not appear to have published any information about the origins of the Mexican strain which was back-crossed with the genetically-modified Rockerfeller strain prior to releases in Cayman and Brazil, and neither the Asian nor Latin American strains of OX513A appear to have been tested for disease transmission properties.

The use of non-native strains in Oxitec's open release experiments, including strains which might in theory be more effective than local strains at transmitting Yellow Fever, raises the possibility that such strains could become established at the release sites and introduce new risks to public health. Whether this is a problem in practice will depend on the disease-transmitting properties of the back-crossed strains and the extent to which the GM mosquitoes may survive and breed (due to the survival of a small percentage of the GM mosquito progeny – which may increase in the presence of tetracycline contamination, or if resistance develops over time). If this is an issue, the risk is likely to be greatest in Brazil where jungle reservoirs of yellow fever still exist and urban yellow fever outbreaks can occur.

Oxitec has provided insufficient information about the mosquito strains it has released and their potential impact on the transmission of viruses including dengue, chikungunya and yellow fever if these strains become established in the wild.

Could resistance develop so that more GM mosquitoes survive and breed, or fitness of the GM mosquitoes is reduced over time?

A key difference between the Sterile Insect Technique (SIT) using irradiated insects and the release of genetically modified (GM) insects is that radiation-induced sterility involves multiple chromosome breaks, whereas the RIDL system relies on a specific genetic modification. Radiation-induced sterility therefore has built-in redundancy that is not provided by molecular genetic approaches.[124] A number of authors have therefore speculated that any genetic or molecular event that allows the GM mosquitoes to survive and breed successfully could therefore be rapidly selected for during mass production.[125] If this happens, the conditional lethality effect could rapidly disappear as resistance develops in production facilities or in the field.

Mutations will occur randomly in any population of mosquitoes. Large-scale production of GM mosquitoes could produce genetic changes that are unexpected and unstable[126,127] although Oxitec has developed a method that it claims improves stability.[128]

Oxitec has published some computer modelling of how resistance to RIDL might develop: whether or not resistance will develop in practice depends on a complex combination of factors.[129] Oxitec accepts that resistance may occur but argues that it could be monitored and detected before a significant loss of efficacy occurred, and a new RIDL line could be substituted. It is not clear how realistic this claim is likely to be in practice.

Genetic changes are not the only mechanisms through which resistance could develop: in a conventional SIT programme in Japan, wild females appeared that were unreceptive to mating with irradiated males.[130]

In a recent study in Mexico, 14% of female *Aedes aegypti* received semen from more than one male over a 48 hour period, increasing the likelihood that mating with a GM male will not prevent them reproducing.[131] However, this study could not distinguish between semen and seminal fluid so the incidence of multiple mating may have been over-estimated.

Mass breeding of mosquitoes will also result in loss of fitness over time (due to inbreeding, known as the "colony effect").[132] Loss of fitness means that fewer males will mate with wild females and effectiveness will be reduced. In the use of irradiated SIT, new wild insects can be added to the colony prior to irradiation in order to increase the fitness. With RIDL, new back-crosses between the parent line of GM mosquitoes and new wild mosquitoes would have to be created periodically and introduced to increase the fitness of the colony.

Even if Oxitec's technology were successful in the short-term, there are a number of ways in which resistance could develop. This would render the technology increasingly less effective and could increase biosafety concerns if more GM mosquitoes survived to breed for multiple generations. Loss of fitness and multiple mating might also reduce effectiveness. In dengue endemic areas, the resulting loss of effectiveness might lead to a rebound in cases of disease if dengue transmission and human immunity were initially reduced by the use of the technology.

Compatibility with traditional control methods?
Continuing to use traditional control methods for mosquitoes could further limit the effectiveness of Oxitec's technology by killing the GM males before they mate with the wild female mosquitoes. This is likely if adult insecticides (adulticides) are used, which is not a routine method of control but is common during epidemics. Adult mosquitoes are also often caught in traps and people may also spray them in their houses or gardens. Removing mosquito breeding sites or using larvicides at the same time as releasing GM mosquitoes would also mean that GM larvae in those breeding sites could play no role in the claimed effect on wild mosquito populations of competition between GM and non-GM larvae. On the other hand, failure to use these control methods – if and when they are effective - may put people at unnecessary risk of dengue or other diseases.

In Malaysia, people were asked not to spray insecticides during Oxitec's experiments, in order not to interfere with the results.[133] In Cayman, high levels of mosquitoes were trapped during Oxitec's first open field trial, apparently without use of any existing intervention to reduce the numbers: in fact Oxitec chose sites where no mosquito control was being implemented in order not to interfere with its experiments.[134] This raises serious ethical concerns about whether people are being adequately protected (using existing approaches) during trials of Oxitec's technology and whether community-based control approaches, focused on removing breeding sites, will be undermined.

Use of GM mosquitoes could also divert resources from other approaches (see alternatives, below). Oxitec's business model relies on repeated payments for ongoing releases of large numbers of GM mosquitoes to suppress the wild mosquito population. [135]

Use of Oxitec's technology could undermine other methods of control, either through diversion of resources, or because it may be necessary to suspend the use of other approaches in order to allow Oxitec's GM mosquitoes to survive and breed.

Will people be bitten by GM mosquitoes, or will they harm other organisms?
Oxitec claims that people will not be bitten by GM mosquitoes because it will release only male mosquitoes, which do not bite. However, sorting of males before release is currently done mechanically and is imperfect: in Cayman about 0.5% of GM mosquitoes were females after sorting, although in the first small trial these were then removed by hand.[136] Further, 3%-4% of the offspring of the GM mosquitoes survive in the lab: half of these will be biting females. If released GM mosquitoes encounter tetracycline in the environment, or if resistance develops over time, a much higher proportion of GM female mosquitoes will survive and breed.

Although these percentages are small, the total number of female GM mosquitoes released, or the number of offspring that survive, could be very large. For example, a new facility in Brazil is expected to produce more than 2.5 million adult GM mosquitoes per week for further trials.[137] If the sorting system was not improved, so 0.5% of these mosquitoes were females, this would amount to 12,500 female GM mosquitoes released each week. Depending how successful the released GM male mosquitoes are at mating, there could be thousands more GM females in the next generation for every week of releases.

The synthetic protein tTA is expressed in the all the cells of the GM mosquitoes and could be present in the saliva of biting females: possible hazards such as allergic reactions in humans bitten by GM female mosquitoes therefore need to be assessed.[138] Although Oxitec states that tTA is not expressed in the saliva of the mosquitoes, it has not published any evidence to support this claim. Animals could also be bitten by surviving GM females, and dead GM larvae or pupae as well as adult GM mosquitoes might be consumed by other species.

Oxitec has published insufficient information to demonstrate that surviving GM female mosquitoes do not pose risks to animals or humans.

Traceability and monitoring

Oxitec argues that its GM insects are easy to monitor and trace because they are genetically engineered to contain a fluorescent marker as well as the RIDL trait. However, experiments in caged trials in Arizona using Oxitec's genetically modified bollworms have shown that the fluorescent trait begins to disappear in this species over a matter of days after they are caught in ovitraps, especially in hot weather. Four tests were done: in Period 1 the marker started to fail in one trap at day 4 after set-up: in Periods 2 and 3 scoring reliability starts to decline from around 10 days; only in Period 4 did the marker show very little degradation throughout its duration (35 days).[139] Mean temperatures were 34.5°C (±5.2°C) in Period 1, 31.8°C (±4.9°C) in Period 2, 28.5°C (±7.6°C) in Period 3 and 19.1°C (±7.9°C) in Period 4 and the authors conclude that temperature seems the most influential factor affecting persistence of the fluorescent marker. If these results also apply to GM mosquitoes, the fluorescent marker may not provide a reliable means to monitor releases, unless traps are checked regularly. The adult life span of *Aedes aegypti* can range from two weeks to a month depending on environmental conditions[140] although the marker seems to disappear only after the insects die in the traps. Areas at risk of dengue are strongly linked to higher temperatures.[141] The GM insects can still be identified by testing their DNA (using the PCR method) if the fluorescent marker is no longer visible, but this depends on researchers realising that there may be problems with the marker. Oxitec did not draw attention to these problems with the marker when it published its results: instead it claimed that the experiments were a great success.[142]

Problems with the fluorescent marker in Oxitec's GM insects in hot weather may mean that open releases are inadequately monitored.

Assessing the potential impacts of releases on a complex system

The mosquito species *Aedes aegypti* is part of a complex system which includes multiple mosquito species, four serotypes of dengue virus and other viruses such as chikungunya and yellow fever, and the humans that they bite. Because this system is not fully understood, there is always a danger that nature will adapt to sustained long-term releases of GM mosquitoes in ways that do more harm than good.

The ecosystems of which mosquitoes are a part are not fully understood and transmission of the dengue virus is complex.[143,144,145,146]

Mosquitoes have many predators which may consume eggs, larvae or adults. For example, several species of small crustacea called copepods consume larvae in pools and ponds[147], as do several species of fish[148], tadpoles and aquatic insects[149]: some of which specialise in consuming particular species of mosquitoes.[150] However, container habitats support smaller populations of fewer species compared with ground pools, implying that aquatic predators may be relatively scarce in locations where *Aedes aegypti* tend to lay their eggs. Predators of adult mosquitoes include bats, birds, dragonflies and frogs, which also eat other insects and are therefore not dependent on mosquito populations. Specific predator species and their relative abundance and interactions will obviously vary considerably in different ecosystems.

Factors that could impact on ecosystems include: the very large increase in adult male mosquitoes during the releases; the large number of dead mosquito larvae and pupae produced by the late lethality effect in the offspring of the mating mosquitoes; the subsequent drop (if achieved) in the wild *Aedes aegpti* mosquito population (and/or fluctuations or increases in populations in surrounding areas); and knock-on effects on other species, including *Aedes aegypti* mosquitoes, human behaviours and immunity, and the transmission of dengue and other viruses.

In the European Union, the release of genetically modified organisms (GMOs) is supposed to be carried out according to the 'step-by-step' principle. This means that the containment of GMOs is reduced and the scale of the release is increased gradually, step by step, but only if evaluation of the earlier steps in terms of the protection of human health and the environment indicates that the next step can be taken.[151] Oxitec has by-passed this procedure by seeking to release its GM insects first abroad (although *Aedes aegypti* is not established in Britain, the company is working on other insects that it could have tested first).

If it was following a step-by-step approach, the company could have conducted and published detailed laboratory experiments on the response of its GM mosquitoes to antibiotics from the tetracycline family and combined this with sampling and environmental monitoring to establish environmental concentrations. Similarly, Oxitec could have conducted more detailed studies in the laboratory and caged field trials to study competition effects between its GM mosquito larvae and larvae from wild *Aedes aegypti* and *Aedes albopictus* populations, and also studied the interactions of the wild species where it planned to make releases. This would have helped it to assess whether competition with wild larvae was really likely to enhance the population suppression effect of releasing its GM mosquitoes, and whether *Aedes albopictus* mosquito numbers might increase in response. It is particularly difficult to understand why Oxitec has moved on to conduct large scale open release experiments in dengue endemic areas in Brazil without first publishing its earlier results, and without any attempt to assess the impacts on disease or to consider potential impacts on human immunity or cross-immunity.

If adverse effects are to be avoided a better understanding of the properties of Oxitec's GM mosquitoes and of how wild mosquitoes behave in the environment is needed before GM mosquitoes are released into the open: including their interactions with other species of mosquitoes, the humans that they feed on, and the viruses they carry.

Regulation, consent and transboundary movements

Oxitec has repeatedly argued that any concerns about its science are a matter for the regulators. Yet it undertook its first open releases in a country with no biosafety law (the Cayman Islands) and its risk assessment for Brazil was not published prior to the releases taking place.

In the Cayman Islands, more than 3 million of Oxitec's OX513A GM mosquitoes were released in late 2009 and in 2010: the first open releases of any genetically modified insects anywhere in the world.[152,153,154] In Malaysia, 6,000 GM mosquitoes were released between 21st December 2010 and 5th January 2011[155,156] and since February 2011, more than ten million GM mosquitoes have been released in Juaziero in Bahia, north eastern Brazil.[157] Two more, larger, trials are now planned to test population suppression of mosquitoes in Brazil and a second state (Tocantins) is expected to be included in the experiments.[158,159]

International transports and releases of genetically modified organisms (also known as living modified organisms, LMOs) are governed by the Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity.[160] Countries which are parties to the CPB must take the necessary and appropriate measures to implement the Protocol, which could include the adoption of biosafety laws and a decision making process which requires risks to the environment, taking into account risks to human health, to be assessed before the first import of LMOs for open releases. Under the CPB, the first export of a given LMO for open release must also be notified to the importing Party and the exporter must provide information as part of the notification, including a risk assessment.

The UK is a Party to the CPB and the relevant requirements are implemented in the EU by Regulation (EC) 1946/2003 on transboundary movement of genetically modified organisms.[161] The Regulation requires that the exporter ensures notification, in writing, to the competent authority of the Party or non-Party of import prior to the first intentional transboundary movement of a GMO intended for deliberate release into the environment. The notification must contain the information specified in Annex I, which includes a previous and existing risk assessment report consistent with Annex II of Directive 2001/18/EC (the EU Directive covering deliberate releases of GMOs) and the exporter is required to ensure the accuracy of the information contained in the notification. The Regulation requires the exporter to send a copy of the notification documents to the competent authority of the Member State from which the GMO is exported and to the European Commission, which must make these documents available to the public in accordance with the Community rules on access to environmental information (allowing for some aspects to be withheld on grounds of commercial confidentiality). The Genetically Modified Organisms (Transboundary Movements) (England) Regulations 2004 implement this EC Regulation in England, making the Department of the Environment and Rural Affairs (Defra) the competent authority.[162]

Table 1 shows the dates of the exports of GM mosquito eggs by Oxitec for open release to the Cayman Islands, Brazil and Malaysia and the dates the notifications were received by Defra (no notifications were sent to the EC directly and it did not receive them until after they had been sent to Defra). To date, no notifications have been received by Defra for exports for open release to other countries.

Table 1: Transboundary notification dates for Oxitec's GM mosquito eggs for open release[163]

Date of export	Date Defra received notification	Date of receipt of notification by importing party	Reported date of first open release	Destination
4.11.09	19.11.10	26.8.09	19.11.09[164]	Cayman Islands
9.2.11	14.2.11	17.12.10	24.02.11[165]	Brazil
15.9.11	16.9.11	20.10.10	21.12.10[166]	Malaysia

Table 1 shows that Defra did not receive notifications for either the Cayman Islands or Malaysia until after the open releases there had taken place. There is some confusion about

the notification in Malaysia because the strain released was developed in Malaysia by crossing Oxitec's OX513A GM mosquitoes with an Asian strain of *Aedes aegypti* at the Malaysian Institute of Medical Research (IMR), resulting in the OX513A(My1) strain which was used in the experiments (discussed further below). In Brazil, open releases were reported on 24th February 2011, only ten days after the notification was sent to Defra. Risk assessments were not published prior to the trials in either Cayman or Brazil.

Cayman Islands

Cayman had adopted no biosafety law at the time of Oxitec's experiments: as a British Overseas Territory the provisions of the Cartagena Protocol had not been extended to the islands.[167]

The existence of the Cayman Island trials was not publicised outside the islands until after the experiments had been completed in November 2010. [168,169] The risk assessment associated with the transboundary notification of the export of eggs, written by Oxitec and dated October 2009, was finally made public in response to UK parliamentary questions on 13th January 2011.[170,171] GeneWatch UK obtained the other transboundary notification documents on 3rd February 2011 following information requests to the European Commission and Defra. An unsigned import permit for 350,000 eggs is dated 28th August 2009: consistent with the date of notification to the importing party given in Table 1, but not with the October 2009 date on the risk assessment (which should have been provided as part of the notification documents, prior to the import permit being granted). GeneWatch raised concerns about this process with Defra and the European Commission in February 2011[172,173] but the only concrete effect was that a reminder was issued to UK biotech companies to comply with the regulations: in addition a belated admission was made (on 9th April 2011) that a transboundary notification for exports to Brazil was needed.[174]

Brazil

The UK parliament was twice misinformed that exports of GM mosquito eggs to Malaysia and Brazil were for contained use only (on 27th January[175] and 28th February 2011[176]). To answer the parliamentary question on 27th January, Defra had sought further information from Oxitec: the company stated that all exports except to Cayman had been for contained use[177]. In fact releases in Malaysia had already taken place and the Brazil releases had already been approved by the Brazilian regulator CTNBio on 17th December 2010.[178] According to Table 1, Defra received the Brazil notification on 14th February 2011, but it continued to claim in its response to the parliamentary question on 28th that exports to Brazil had been for contained use only. As a result, no risk assessment was made available for the Brazil trials in the UK until months after the experiments had started.

Brazil is a Party to the CPB and has adopted a biosafety law, but the risk assessment was kept confidential at the request of Oxitec's partner Prof. Margareth Capurro at the University of São Paolo.[179] Experiments in Brazil followed from a meeting organised by UK Trade and Industry (UKTI) on 25th April 2007, between the UK Foreign and Commonwealth Office (FCO), Oxitec's CEO and Head of Public Health, the Technical Director of the Brazilian Institute of Molecular Biology, the Head of Technology & Innovation at Fiocruz (the Oswaldo Cruz Foundation, under the Brazilian Ministry of Health) and the Coordinator for Biotechnology at ABDI (the Brazilian Agency for Industrial Development). It was agreed at this meeting that Oxitec and Fiocruz should initiate a collaboration to evaluate Oxitec's technology in the field in Brazil and that "*Brasil's current GM regulations are unlikely to hamper or slow down this step*".[180] It was also agreed that Fiocruz may also be interested in licensing the technology for commercialisation in Brazil, and possibly other countries as well, and that implications of Brazil's current and proposed GM regulations on future commercialisation would be studied by both parties in the coming months. Further emails provided to GeneWatch UK as a result of Freedom of Information requests show that

Oxitec's technology was identified by UKTI as one that could be showcased as part of an attempt to encourage venture capital investment and commercialisation of patented British biotechnologies in Brazil. The documents refer not only to GM mosquitoes but also to potential use of GM agricultural pests in future (Mediterranean fruit fly and codling moth).

Open releases of Oxitec's GM mosquitoes were discussed at a meeting in November 2010 as part of a collaboration between Oxitec, the not-for-profit company Moscamed and Prof. Margareth Capurro at the University of São Paolo[181] prior to approval by CTNBio on 17th December. In February 2011, the first phase of the releases was carried out: the second phase started at the end of April with larger releases and the numbers increased further from July.[182] GeneWatch UK made information requests to Defra and EC for the transboundary notification documents on 20th April 2011, after Defra had conceded that a notification was needed: we received a heavily redacted copy of the notification documents on 4th August 2011 and (following appeal) a similar set of documents, with minor changes in redactions on 23rd November 2011. The original redacted documents were also released to the UK parliament following a parliamentary question on 2nd November 2011.[183,184] The first 18 months of open releases covered by the risk assessment have now been completed.

Malaysia

Malaysia is a Party to the CPB and has adopted a biosafety law. Unlike Cayman or Brazil, Malaysia did publish its own summary risk assessment prior to authorising releases of GM mosquitoes.[185,186,187] The risk assessment was made available on the Biosafety Clearing House of the Cartagena Protocol on Biosafety and in addition the application dossier for Malaysia was made available for scrutiny by local stakeholders on request and by appointment. Nevertheless, the process was criticised for a number of reasons, including limited public access to the application document, and lack of transparency when the trial actually took place.[188,189] The decision was the first approval for a field trial of any GMO made under Malaysia's biosafety law.[190] There is some confusion about the transboundary notification in Malaysia because the strain released was developed in Malaysia by crossing Oxitec's OX513A GM mosquitoes with an Asian strain of *Aedes aegypti* at the Malaysian Institute of Medical Research (IMR), resulting in the OX513A(My1) strain which was used in the experiments. The notification documents (released to the UK parliament on 14th November 2011 in response to a parliamentary question) include a shipping schedule for 100,000 OX513A GM mosquitoes from Oxitec to the IMR dated 15th September 2011, presumably intended to be used in the second experiment scheduled for an inhabited area (which has not yet taken place). [191,192] The risk assessment included with the notification documents is the same summary assessment already published in Malaysia, which covers the release which has already taken place, plus a second small-scale release in an inhabited area. However, the dates in Table 1 suggest that a notification was made by Oxitec to Malaysia prior to the first experiment (on 20th October 2010) but not copied to Defra. As noted above, the UK parliament was twice misinformed that exports of GM mosquito eggs to Malaysia and Brazil were for contained use only (on 27th January[193] and 28th February 2011[194]), after the first open release experiment in Malaysia had already taken place.

Other countries

Oxitec informed Defra on 24th January 2011 that it had made exports for contained use to a number of countries, with the date of the first export for contained use being: Malaysia (22 Nov 2006); Brazil (20 Oct 2009); France (greater than five years ago); India (30 Sept 2008); Singapore (16 Nov 2010); Thailand (06 May 2008); United States (7 Sept 2007); Vietnam (23 Nov 2009).[195]

Oxitec has established partnerships in Panama (with the Gorgas Memorial Institute[196,197]), India (with Gangabishan Bhikulal Investment and Trading Limited, GBIT[198]) and the USA (Florida Keys Mosquito Control District[199]) with a view to making open releases in those

countries in the future. Exports to France were for contained use only and reports from Vietnam state that the country does not intend to make open releases of Oxitec's GM mosquitoes.[200] Proposed releases in the USA (Florida Keys) have been put on hold until it is clear how they would be regulated: the US Food and Drug Administration (FDA) is currently considering an application.[201,202] The US is not a party to the Cartagena Protocol on Biosafety and the lack of a coherent regulatory regime for GM insects has been criticised.[203] It is unclear whether Singapore and Thailand are still considering using Oxitec's technology. A recent report suggests that releases are also planned in Sri Lanka.[204]

Standards and content of the risk assessments in Cayman, Malaysia and Brazil

Annex 1 of Regulation (EC) No. 1946/2003 (which implements the requirements of the CPB in the EU) specifies that the exporter must supply a risk assessment with the notification documents that meets EU standards. According to the European Commission it is for the company to self-certify that the risk assessment meets EU standards and no oversight of this is necessary.[205] In the case of Malaysia and Brazil, risk assessments by the exporter have still not been provided: only a summary assessment by the Malaysian advisory committee (GMAC)[206] and a submission to the Brazilian regulators by the University of São Paulo[207]. In the case of Cayman, a risk assessment made by Oxitec was eventually provided (after the experiments were finished). [208]

These risk assessments do not address many of the issues outlined above:

1. Survival of GM mosquitoes in the presence of tetracycline contamination was raised by Third World Network during the consultation process in Malaysia.[209] A workshop of experts held in India also highlighted this concern.[210] The Malaysian regulators imposed a requirement on local authorities to document the presence or otherwise of aquaculture, poultry and pharmaceutical industries within a vicinity of 500 meters of the release sites, and information on whether any of these industries regularly use tetracycline in their operations.[211] However, at the time the information about the 15% survival rate of the GM mosquitoes when fed on cat food was not publicly available, partly due to delays in releasing the transboundary notification documents and partly because many of the released documents included significant redactions due to claims of commercial confidentiality.[212] It is unclear whether regulators were aware of the 15% survival rate, which was reported in a laboratory protocol. In January 2012, Professor Mumford of Imperial College London, who collaborates with Oxitec as part of the Mosqguide project[213], responded to publication of this information by NGOs by saying that risk management should take account of levels of tetracycline in the environment.[214] The issue of tetracycline contamination was argued not to be a problem in the risk assessments for Cayman and Brazil and no conditions were applied.
2. Potential negative impacts of releases on mosquito populations and disease incidence and questions about the effectiveness of population suppression for mosquitoes were raised in a letter from GeneWatch UK to the Malaysian Genetic Modification Advisory Committee (GMAC) in January 2011.[215] The GMAC replied that these issues were not relevant to the limited trial conducted in Malaysia to date but would be considered prior to commercial releases.[216] The Malaysian authorities conducted its small-scale trial in an uninhabited area and required that the area be certified free of dengue for three months prior to the trial.[217] None of the issues that could result in increased transmission of dengue fever were discussed in the risk assessments made for the larger trials in inhabited areas in Cayman Islands or Brazil. This is of particular concern in Brazil where dengue is endemic and where larger-scale releases are now planned in inhabited areas with a view to suppressing *Aedes aegypti* populations, especially as preliminary results suggest that population suppression requires very high release ratios and is therefore likely to be of limited effectiveness.[218] Only sporadic cases of dengue occur in the Cayman Islands,

although it is unclear whether checks were made to ensure the area was dengue-free at the time of the experiments[219].

3. A workshop of experts held in February/March 2010 in India highlighted the potential for multiple mating to affect the efficacy of the programme in an article published in June 2010[220] (in a journal guest edited by Oxitec's SS Vasan[221]). None of the risk assessments have considered this: it remains unclear whether this issue is important.
4. Impacts on other mosquito species were considered during the expert discussions in Malaysia and rated as a 'medium' risk.[222] This issue was highlighted by the Malaysian authorities as one which would need to be addressed prior to any large-scale releases.[223] The Cayman risk assessment states that in 2007 the levels of *Aedes albopictus* were accounting for approximately 5% of the larval finds on the island: it states that an increase in *Aedes albopictus* is not of concern for a limited duration trial as ecological niche replacement is only likely to happen over an extended time period and could be monitored. However, it is questionable whether this claim is compatible with the rapid establishment of *Aedes albopictus* in most of Florida and in Bermuda. The possibility of an increase in *Aedes albopictus* mosquito populations was not considered in the risk assessment provided for Brazil.
5. The likely release and/or survival of some female GM mosquitoes that bite has been raised in a journal paper by scientists based at the Max Planck Institute.[224] The Malaysian authorities required that the monitoring period was extended and additional fogging done to seek ensure that there would be no residue GM mosquitoes in the environment: Malaysia also identified sorting error as an issue that would have to be addressed if large quantities of GM mosquitoes were to be released, and required a manual recheck of all sorted males.[225,226] The conditions applied in Malaysia were not applied in Cayman or Brazil. This is a particular concern in the light of the large numbers of GM mosquitoes released in Brazil (reportedly 10 million) and the plans for further, larger trials.
6. The potential development of resistance has been raised in a journal paper by scientists based at the Max Planck Institute.[227] The Brazil risk assessment states that the strain has been shown to be stable for 60 generations and has been produced on a large scale in the lab without any problems. However, this does not really address the issue of what will happen in large-scale production, or whether there will be adaptations in the field. If this is an issue, it is particularly relevant to the large-scale releases now planned in Brazil.
7. Concerns that more complex poorly-understood effects could arise, such as a new strain transmitting more serious diseases, were raised by an anonymous expert in the Malaysian press.[228] None of the risk assessments consider any possible adverse effects on the transmission of viruses.
8. In Malaysia, tests were done on a single predator to see if consuming larvae had any harmful effects on it.[229] In the Brazilian risk assessment it was claimed that there were no predators other than some lizards and spiders for which the mosquitoes formed only a small part of the diet: no tests were reported. The Cayman risk assessment lists some relevant species, such as bats and snakes, and mentions predators such as dragonflies and spiders, but refers to no analysis.
9. The need to establish an effective Integrated Pest Management (IPM) system to incorporate the new GM technology was highlighted as an important requirement before undertaking large-scale releases in Malaysia.[230] Other control methods in use are listed in the Cayman risk assessment, but no analysis of their compatibility with RIDL is discussed.
10. The fact sheet published for the field trials in Malaysia states that the fluorescent marker allows the GM mosquitoes to be easily identified in the laboratory and field.[231] The recently published experiments with bollworms described above appear to suggest that this may not be correct, at least if the temperature is high and traps are not checked regularly. The preliminary results from Brazil rely on the fluorescent

marker[232] as do some of the findings reported from the first stage of the experiments in Cayman.[233] There is no reference in any of the risk assessments to potential problems with the marker.

11. In Malaysia a local strain was developed by backcrossing the genetically modified Rockerfeller strain with a Malaysian strain of *Aedes aegypti*. This was tested for insecticide resistance, but no tests on disease transmission have been reported although the strain will not be exactly identical to a native strain. In Cayman, Oxitec released the GM Rockerfeller strain back-crossed for several generations with a Mexican strain, rather than a local strain, and this also appears to be the case in Brazil. The Cayman Islands risk assessment states that the OX513A strain has been assessed for insecticide resistance to current control insecticides and no significant resistance was detected from bioassays or molecular analysis: but the risk assessment does not describe tests for disease transmission. Release of non-native strains and failure to test for disease transmission could be a serious omission because of the risk that a non-native strain may become established at release sites. For comparison, in the UK, Oxitec has been prevented from releasing a GM Diamond Back Moth (an agricultural pest) because of concerns about the use of a North American background strain, which is subject to controls under plant pest control regulations.[234]

It appears unlikely that the risk assessments which accompanied the transboundary shipments of Oxitec's GM mosquito eggs to the Cayman Islands, Malaysia or Brazil would be judged to meet EU risk assessment standards by any independent body. The requirements in Annex II of Directive 2001/18/EC include direct and indirect interactions between the GMO and target and non-target organisms[235] (including competitors, prey, hosts, symbiots, predators, parasites and pathogens) and human health: none of which have been thoroughly considered. A critique of the Cayman and Malaysian risk assessments has been published in the scientific literature: together with a useful check list for assessing the scientific quality of approvals.[236] The authors conclude that there are deficiencies in the regulatory process and significant omissions in the information made publically available prior to releases in the Cayman Islands and Malaysia. Draft guidelines for risk assessment of GM insects in the EU have recently been published for consultation by the European Food Safety Authority[237], following the publication of an expert report[238]. The draft guidance is likely to be controversial and to require significant revision following the consultation process.[239]

It is widely recognised that informed consent from any person potentially affected by the release of transgenic insects (including children) is important for the ethical conduct of trials.[240] 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).[241] In the absence of any published risk assessment it is hard to see how consent in Cayman or Brazil could be regarded as fully informed.

In Cayman, the only public information that appears to have been provided was a video entitled "MRCU sterile mosquitoes", which does not mention that the mosquitoes are genetically-modified and also repeatedly (incorrectly) refers to them as sterile.[242] The video was put on the Cayman Islands Government Information Service (GIS) website on 4th October 2010, after the releases had already taken place.[243] A press article in the Cay Compass on 2nd October 2009 stated that experiments with GM mosquitoes were planned but that a decision had not yet been made to go ahead and would be subject to budget concerns, logistics and priorities, and a permit from the Department of Agriculture. Open releases began on 18th November[244], with no further press reports. This lack of consent or published risk assessment for the Cayman trials has attracted strong criticism from both scientists and NGOs.[245,246]

In Brazil, Oxitec's partners held public meetings, went door-to-door, communicated via local media (radio, TV and press), community meetings, printed information (posters and leaflets), school presentations, carnival parades and use of trucks with loudspeakers and employed a journalist to promote the experiments.[247] However, lack of publication of the risk assessment and the many issues that were unaddressed means that people were not fully informed of the pros and cons of the experiments. The Brazilian press reported that many residents of Itaberaba had not realized that the neighbourhood had become a kind of open-air laboratory for Oxitec and despite the information work, few knew what the scientists were doing there.[248] There has also been criticism from scientists of the releases in Brazil.[249]

The Malaysian authorities appear willing to improve the risk assessment and consultation process, and seek informed consent before considering whether to conduct further trials in future.[250] They have highlighted additional issues that will need to be addressed before large-scale releases could take place (these are: release of transgenic mosquitoes may cause other pests to become more serious; increase in the population of another mosquito species due to suppression of the target mosquito; stability of the transgenes in the field; behaviour of GM mosquitoes in the field; sorting error when handling large quantities of GM mosquitoes to be released; and establishment of an effective Integrated Pest Management system to incorporate the new GM technology). It is currently unclear if or when further releases will take place and the potential for alternative approaches has begun to be debated.[251]

However, in Brazil, mass production of GM mosquitoes is being scaled up in preparation for further experiments, with construction of a new facility in Brazil to produce more than 2.5 million adult GM mosquitoes per week (despite the poor results of the experiments to date).[252] No risk assessment for these experiments has been published yet.

In summary, only Malaysia published and consulted on a summary risk assessment prior to releasing 6,000 GM mosquitoes into the open in an uninhabited area. In Cayman and Brazil, no risk assessment was published despite much larger numbers of mosquitoes being released in inhabited areas: this is a particular concern in Brazil where dengue is endemic. The risk assessments associated with the transboundary notifications of exports of GM mosquito eggs from the UK were not publicly available until after the experiments were underway (or in some cases, completed) and do not meet the necessary standards or requirements.

Alternatives

There are a wide variety of alternatives to the use of GM mosquitoes as a method to tackle dengue, although all have some costs, difficulties or limitations and/or carry some potential risks.

Current methods of mosquito control include[253]:

- Destruction of breeding sites by government-employed inspectors or local communities;
- Killing of larvae using a variety of larvicides;
- Environmental measures, such as improving water and sewage systems and shredding waste tyres (which provide potential breeding sites);
- Killing of adult mosquitoes using fogging with insecticides (adulticides) inside houses, or more widely (e.g. through aerial spraying) on an occasional basis when numbers are high.
- Educating the public concerning mosquito habits and ways individuals can protect themselves from mosquito attack.

Absence of a tap water supply is correlated with an increased incidence of dengue, because water storage containers used by households without tap water supply provide mosquito breeding sites.[254] A newspaper article in Brazil includes criticism from local residents about trials of Oxitec's GM mosquitoes in their area. Housewife Maria da Gloria Pinheiro says: "*I know that if we had water on tap, things would be very different. No standing water, which we need for the basics, such as is doing laundry and preparing food. (With water on tap) we would not have mosquitoes, either with dengue or without*."[255] However, piped water supplies need to be reliable, otherwise residents must continue to store water to guard against interruptions in supply.

A study in Brazil compared two neighbouring cities, Rio de Janeiro and Niterói, which have similar climates, populations and environments likely to contribute to elevated *Aedes aegypti* infestation rates. The authors reported that Rio had twice the dengue incidence of Niterói, which they attributed to significant differences in public health coverage.[256] They conclude that the problem with dengue in Rio is the result chaotic urbanization combined with a poor primary-care system.

Preventing dengue not only prevents suffering and death but also avoids the economic costs associated with healthcare and with absenteeism from school and work.[257,258,259,260]

Community based approaches to reducing mosquito populations have been shown to be a cost-effective way to control *Aedes aegypti* populations, producing sustained health benefits and economic savings,[261,262] although, in Singapore, a rebound effect in dengue cases occurred over time. [263] A major focus is on removing mosquito breeding sites through household level control, combined with other methods (larvicides and selective use of adulticides). Examples of methods under development or being tested include the use of insecticide treated curtains and water container covers tested by WHO/TDR[264]; a variety of newly developed larvicides, including e.g. potash alum[265], and various kinds of mosquito traps.[266] A community-based program for dengue control using local predators (tiny freshwater crustaceans called *Mesocyclops*) reported some success in Vietnam in 2000-2003 and this programme has been successfully expanded.[267,268] A combination of different methods can be focused on areas most at risk using monitoring and surveillance with Geographical Information Systems (GIS): this approach has successfully suppressed dengue transmission in a study in Thailand.[269,270] Improved methods of early diagnosis of infection could also help to reduce the spread of dengue outbreaks by allowing early action.[271,272]

Studies such as these suggest that there is no shortage of currently available methods to reduce the numbers of mosquitoes, the incidence of biting, or the spread of the disease, but there is often a lack of political will or available resources to implement these measures in an effective way. Further, the success of different strategies will depend on local conditions: for example, the successful use of biological control in Vietnam is a result of most breeding sites being in relatively large containers, which is not always the case elsewhere. As long as dengue persists at high levels in dengue endemic countries, sporadic cases will also continue to occur in countries where *Aedes aegypti* or *Aedes albopictus* mosquitoes exist, because travellers will occasionally arrive with dengue fever which may then be transmitted locally by these mosquitoes.

It is of course unlikely that any mosquito control measure will be 100% effective and the development of vaccines and new treatments is therefore also important. A number of vaccines are currently under development: one is in phase III trials (the final phase of clinical trials) and a production facility is under construction in France.[273] If the trials are successful, the vaccine could reportedly begin to be introduced within three to five years, although

access and pricing are currently unclear.[274] A study in Brazil has suggested that a dengue vaccine could be produced at an affordable price.[275]

Another approach which is being researched involves infecting *Aedes aegypti* with bacteria called *wolbachia*, which reduce their ability to transmit disease.[276,277] Neither the mosquitoes nor bacteria used in this approach have been genetically modified: however, there is a need for careful assessment of any biological control method.

Many alternatives to the use of Oxitec's GM mosquitoes exist or are under development. All alternatives have pros and cons but some methods are already demonstrably more effective than Oxitec's approach appears to be. If a vaccine becomes available in a few years this could complement existing public health approaches to reducing mosquito populations. More fundamental changes, such as improving water supplies and healthcare, play an essential part in reducing the incidence of dengue.

Conclusions

Oxitec has promoted a PR message which over-simplifies the complex relationship between multiple species of mosquitoes, the viruses they carry and the humans that they bite. The company claims that there is no risk to its experiments because (1) no GM mosquitoes will survive and (2) if anything goes wrong the system will simply return to how it was before the GM mosquitoes were released. Neither of these claims is likely to be true.

Release or survival of some biting female GM mosquitoes is almost inevitable and, over time, irreversible effects on ecosystems could occur. Reliance on the antibiotic tetracycline as a chemical switch for the 'conditional lethality' genetic trait is a fundamental flaw because tetracycline is widely used in medicine and agriculture.

The most likely outcome of Oxitec's experiments is that they fail to make any impact on highly complex and mobile mosquito populations or the incidence of dengue fever. However, there is also a risk that partial or temporary suppression of mosquito populations in dengue endemic areas has adverse impacts on the transmission of the disease. There may be a risk of increasing *Aedes aegypti* mosquito populations in areas surrounding the release sites, or a rebound effect on mosquito populations; an increase in more serious cases of disease due to partial suppression of mosquito populations; and/or an increase in the risk of dengue due to reducing other methods of control. The likely impact of the releases on mosquito populations and disease incidence is poorly understood and these potential adverse consequences have not been considered in the risk assessments.

Evidence of limited efficacy of Oxitec's approach to date should be taken very seriously, because it is well known that any approach that is of limited or temporary efficacy can have adverse impacts on disease incidence or severity in dengue endemic areas, putting people's health at unnecessary risk.

If short-term success in repressing mosquito populations is achievable this could be followed by long-term adaptions that make the dengue problem worse or lead to other unintended consequences. The evolution of resistance, loss of fitness, multiple mating, or breeding in the presence of tetracycline contamination, might cause populations to increase again, because incomplete sterility or loss of mating fitness is expected to undermine any population suppression effect caused by the releases. Increasing numbers of GM mosquitoes might survive and reproduce, including biting GM females. Such built-in long-term failure could lead to a rebound in cases of disease (over and above what might occur without any intervention) due to effects on human immunity in dengue endemic areas. There is considerable uncertainty about long-term effects: for example, other species of mosquitoes (particularly *Aedes albopictus*) could increase in numbers if populations of

Aedes aegypti fall; complex ecosystem interactions will occur; and, in the longer term, there is a possible but poorly investigated risk that dengue or other viruses evolve to become more virulent.

It is not clear who would be liable for any long-term negative effects.

Only Malaysia held any kind of consultation and published a risk assessment prior to conducting open release trials using Oxitec's GM mosquitoes: to date Malaysia has conducted only a relatively small trial in an uninhabited area. Nevertheless, many issues remain to be addressed and only a summary of the risk assessment was published. In the Cayman Islands and Brazil, regulators have not allowed any independent scrutiny of risk assessments, and as a result the approvals process for open trials has been seriously inadequate. Trials in Cayman and Brazil went ahead in inhabited areas without the fully informed consent of local people. Oxitec failed to notify the UK and EU authorities of its shipments of mosquito eggs in a timely way, with the result that the risk assessments were not available for public scrutiny in the exporting country until long after open releases had begun: these assessments would be highly unlikely to be judged to meet EU risk assessment standards by any independent body.

The timetable for the releases, including a push to commercialise Oxitec's technology, appears to be driven by the needs and wishes of the company's venture capital investors[278,279] rather than any serious consideration of the pros and cons, or of alternatives. The UK Government has promoted the technology heavily via UK Trade and Investment (UKTI) as part of an economic strategy designed to boost exports of patented biotechnologies overseas[280] and has changed tax rules for venture capital to help fund the company.[281] This has resulted in a political agreement with Brazil to test and commercialise the technology, apparently without any independent scientific or public scrutiny of risk assessments or preliminary results. It is questionable whether this approach is in the best interests of people living in areas at risk for dengue fever.

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