Failures of the transboundary notification process for living genetically modified insects

GeneWatch UK

August 2014

GeneWatch UK is a not-for-profit organisation which seeks to ensure that genetic science and technologies are used in the public interest. We support a precautionary approach to releases of living modified organisms (LMOs) into the environment. GeneWatch UK has made extensive investigations of UK company Oxitec's exports of living genetically modified (GM) mosquito eggs to the Cayman Islands, Malaysia, Brazil and Panama, in order to undertake open release experiments. We have major concerns about poor standards of risk assessment and lack of timeliness, transparency, public consultation and informed consent to these experiments.

Oxitec's releases of GM mosquitoes in the Cayman Islands and Malaysia have ceased but open releases in Brazil have continued since 2011 and started in Panama in 2014. Brazil's GM regulator has also approved commercial releases of GM mosquitoes, although no results of the trials have yet been published. We are deeply concerned that:

- Neither Brazil nor Panama has submitted any information to the Biosafety Clearing House in relation to genetically modified mosquitoes (breach of Article 20);
- The transboundary notification from export of GM mosquito eggs by Oxitec to Panama contains no risk assessment and written informed consent of the Party of import was not received prior to export (breach of Article 8 and the requirements of Annexes I and III);
- For both Panama and Brazil, the Party of Export (the UK Government) has failed to ensure that the exporter meets the necessary legal requirements for accuracy of information, or to ensure that risk assessments meet the necessary standards (breach of Article 8);
- The Parties of import (Panama and Brazil) have failed to ensure that risk assessments undertaken pursuant to the Protocol are carried out in a scientifically sound manner (breach of Article 15).

Because no published, comprehensive and reliable risk assessments exist, local people in the areas of release have not been provided with any information about the risks and have been unable to give their fully informed consent to the experiments.

We are deeply concerned that these failures effectively render the Protocol useless as a means to fulfil its objective of protecting biological diversity, taking account risks to human health (Article 1). We urge the Secretariat and Parties to take urgent action to rectify this situation and restore the credibility of the Protocol.

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. Releases of many millions 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 *Aedes aegypti* which transmit the tropical disease dengue fever. There is as yet no evidence from any country that releases of GM mosquitoes can reduce the incidence of dengue fever.

Oxitec's genetically modified (GM) mosquitoes fall within the definition of living modified organisms (LMOs) under the Cartagena Protocol on Biosafety (CPB). The UK, Panama, Brazil and Malaysia are all Parties to the Cartagena Protocol and have adopted relevant biosafety laws. The Cayman Islands, a British Overseas Territory, were not a Party at the time Oxitec conducted its releases of GM mosquitoes there. Malaysia is the only country to have submitted any information to the Biosafety Clearing House (a summary of its risk assessment) and has since ceased to allow releases. The focus of this briefing is therefore on Brazil (which began open releases in 2011) and Panama (which began open releases in 2014).

Legal requirements

The relevant legal requirements for export are implemented in the UK through Regulation (EC) 1946/2003 on transboundary movement of genetically modified organisms.[1] Section 1 of Chapter II covers exports of GMOs to third parties for deliberate release into the environment. Article 4 requires the exporter to ensure 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 shall contain, as a minimum, the information specified in Annex I, which includes a previous and existing risk assessment report consistent with Annex II of Directive 2001/18/EC. Article 5 specifies that no first intentional transboundary movement may be made without prior written express consent of the Party of import. Article 6 requires a copy of the notification documents to be sent to the competent authority of the Member State from which the GMO is exported and to the Commission. Without prejudice to Article 16 (which allows some information to be kept commercially confidential), the Commission shall make these documents available to the public in accordance with the Community rules on access to environmental information. Under Article 11, these provisions do not apply to transboundary movements of GMOs intended for contained use rather than deliberate release.

Regulation (EC) No 1946/2003 requires that the environmental risk assessment (ERA) provided by the exporter meets the standards of EU rules on risk assessment contained in Directive 2001/18/EC[2]. For GMOs which are not plants, a list of issues that must be covered by the risk assessment is included in Annex II, D.1 of the Directive. 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).[3] Pages 73 to 107 of the EFSA Guidance provide details on the following specific areas of risk for GM insects:

- Persistence and invasiveness of GM insects, including vertical gene transfer (VGT);
- Horizontal gene transfer;
- Pathogens, infections and diseases;
- Interactions of GM insects with target organisms;
- Interactions of GM insects with non-target organisms (NTOs);

- Environmental impacts of the specific techniques used for the management of GM insects;
- Impacts of GM insects on human and animal health.

The Genetically Modified Organisms (Transboundary Movements) (England) Regulations 2004 implement Regulation (EC) 1946/2003 in England. This designates the Secretary of State for Defra as the Focal Point and Competent Authority for the purpose of the Council Regulation. The Secretary of State shall enforce and execute the provisions of these Regulations and the specified Community provisions, or direct the relevant local authority to do so, or act jointly with the local authority to enforce and execute the provisions and appoint inspectors with rights of entry to inspect premises and require the provision of information. The Secretary of State may also serve notice in writing to obtain information. It is an offence to fail to comply, fail to provide information, or to make false entries in records and failure to comply may lead to a fine or imprisonment.

Other risk assessment guidance

EFSA's risk assessment Guidance is directly relevant to any export of GM mosquitoes from the UK, since Regulation (EC) 1946/2003 requires the exporter to meet EU standards. However, other guidance also exists. Under the CPB, the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management has produced Guidance on the Risk Assessment of Genetically Modified Mosquitoes.[4] In addition, relevant academic papers which discuss the risk assessment of GM insects, including GM mosquitoes, include Reeves et al. (2012)[5] and David et al. (2013)[6].

Panama: Oxitec's failure to comply with the requirements

Panama began open release experiments using Oxitec's GM mosquitoes in April/May 2014. Panama has provided no information to the Biosafety Clearing House regarding this decision.

GeneWatch UK has repeatedly asked the UK Department for the Environment, Food and Rural Affairs (Defra) for a copy of the risk assessment which UK company Oxitec was required to provide to the Panamanian authorities under Regulation 1946/2003/EC prior to exporting GM mosquito eggs to Panama for open release. We made our first request for the notification documents on 14th January 2014, following reports that an open release of Oxitec's GM mosquitoes had been approved by the Panamanian authorities. We received a copy of the notification documents on 10th February 2014, but these did not include a copy of the risk assessment, which should meet EU standards (Regulation 1946/2003/EC Article 4 and Annex 1), or a copy of the prior written informed consent of the Party of Import (required under regulation 1946/2003/EC Article 5). Defra has since informed us that these documents do not exist. This is a clear breach of Regulation 1946/2003/EC and the Cartegena Protocol itself:

- The decision to import is distinct from the decision to release and requires a specific procedure to be followed, consistent with Regulation 1946/2003/EC which implements the Cartagena Protocol;
- Failure to provide a risk assessment by the company means that the Panamanian authorities and public have no means to hold Oxitec to account for the information it provides in its role as an exporter: an informal process of information gathering cannot substitute for this;
- Failure to provide a risk assessment which meets EU standards means that the public in Panama may be exposed to unnecessary and unacceptable risks to human health and the environment;

• Failure to provide the risk assessment means that this is unavailable to civil society organisations and independent experts on request, thus there is no mechanism for scrutiny of whether or not the risk assessment meets the required legal standards.

In Panama, the Gorgas Institute, which acts as Oxitec's partner for the experiments, also has responsibility for supervising, regulating and controlling the risks of GMOs to health, on behalf of Panama's Department of Health. The Institute has produced a risk assessment which is clearly marked "*Uso confinado*" (confined use). This risk assessment does not meet EU or international standards for open release of GM insects.

The lack of an adequate risk assessment means local people were not able to give their fully informed consent to the experiments.

Brazil: Oxitec's failure to comply with the requirements.

Brazil began open release experiments using Oxitec's GM mosquitoes in February 2011. Despite repeated requests for the transboundary notification documents, GeneWatch UK did not receive a (redacted) copy of the risk assessment until 4th August 2011 and (following appeal) a similar set of documents with minor changes in redactions on 23rd November 2011. The risk assessment included in the documents was produced by Oxitec's partner the University of São Paulo, not by the exporter. This is in breach of the requirements in Regulation 1946/2003/EC. The risk assessment omits most of the issues required to be covered under Directive 2001/18/EC. Brazil has failed to send any documentation to the Biosafety Clearing House, although it has since allowed larger-scale experiments to be conducted and the Brazilian regulator CTNBio has approved commercial releases of Oxitec's GM mosquitoes.

Risks

A number of important risks have been neglected due to the failure to provide comprehensive and reliable environmental risk assessments.

1.1. ***Impact on other (non-target) mosquito populations***

Releases of Oxitec's GM *Aedes aegypti* mosquitoes are intended to suppress the wild population of *Aedes aegypti*. Unlike removing breeding sites or using larvicides, this is a single-species approach which does not reduce populations of non-target species. One important question for the risk assessment is whether *Aedes albopictus* (Asian Tiger) mosquitoes, which also transmit dengue and several other viruses (including chikungunya), will increase in numbers and perhaps establish in new areas as a result of competitive displacement of one species by another.

The AHTEG Guidance includes, as an issue for consideration in the ERA: "*Whether, in the absence of the target mosquito, niche displacement by other disease vector species may occur, and if so, whether that can result in an increased incidence of the target disease or other diseases in humans or animals*" (page 47).

The EFSA Guidance states: "*Considering the aim and type of GM insect releases, and also accounting for possible accidental releases, potential impacts on NTO [non-target organisms] that may cause adverse effects include:…(b) a change in abundance or species composition of competitors (e.g. insects exploiting the same ecological niches) of GM insects and the ecological functions they provide*" (p.94) and adds "*Other pest species (e.g. secondary pests) might exploit the available resource and build up high populations which might have an adverse effect on the environment and on human health*" (p.98).

David et al. (2012) state that one issue for consideration is that: "*An initial increase (or decrease) in population size during the transitory state may suppress or displace (or release) a competitor species*".

The risk that numbers of *Aedes albopictus* could increase due to reduced competition for breeding sites and food 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.[7]

In a draft risk assessment submitted to regulators in the USA Oxitec states (page 25): "*It is not clear to what extent Ae. albopictus could or would expand its range into areas currently dominated by Ae. aegypti but it is reasonable to expect a degree of such expansion if no countervailing activities are undertake*n".[8] Oxitec has also published a paper which uses computer modelling to show how *Aedes aegypti* and *Aedes albopictus* may interact.[9] The authors acknowledge that this could have important consequences for the persistence of disease.

Benedict et al. (2007) report that *Ae. albopictus* (a native of Asia that has spread around the world) was established in Panama in 2002.[10] Researchers at Panama University have described *Aedes albopictus* as more dangerous than *Aedes aegypti* and regard it as a more invasive species which may be very difficult to tackle if it moves into an area.[11] In Brazil, both *Ae. aegypti* and Ae. *albopictus* play a role in transmission of the chikungunya virus.[12] The two species have overlapping habitats and sometimes co-exist.[13] *Aedes albopictus* has been responsible for concurrent epidemics of dengue and chikungunya in Gabon,[14] for an outbreak of dengue fever and dengue haemorrhagic fever in Dhaka, Bangladesh,[15] and for the re-emergence of dengue in southern China.[16]

Oxitec frequently cites a review by Lambrechts et al. (2010) to support its claim that *Ae. albopictus* is a less effective vector of dengue than *Ae. aegypti*. However this paper also warns that it is not possible to predict the epidemiological outcome of competitive displacement of *Ae. aegypti* by *Ae. albopictus* and warns that vector status is a dynamic process that in the future could change in epidemiologically important ways.

In the Philippines, Duncombe et al. (2013)[17] suggest that increased numbers of *Ae. albopictus* mosquitoes in vegetative areas later in the wet season may extend spatial and temporal opportunities for dengue fever transmission, which would not be possible if *Ae. aegypti* were the sole vector. They also note that increasing co-circulation of dengue fever virus serotypes in human populations with specific herd immunity may increase the incidence of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are more severe forms of dengue fever resulting from secondary infection with a different serotype. In Sri Lanka, Sirisena and Nordeen (2014) find that the role of *Ae. albopictus* has been underrated and this species is likely to play an important role in the maintenance and transmission of the virus.[18] The greater susceptibility of *Ae. albopictus* to infection is believed to have led to greater dengue virus adaptation, thus Sri Lanka as a whole may be at serious risk of multiple dengue fever/DHF outbreaks in the future with the evolution of new virus strains.

Recently, Grardet al. (2014) identified the presence of ZIKV (Zika virus) in the invasive mosquito *Aedes albopictus* in Gabon and raised the possibility of a new emerging threat to human health.[19]

The risk that *Aedes albopictus* mosquitoes increase in numbers or establish in new areas as a result of the proposed releases has not been considered in risk assessments in either Panama or Brazil. Nor has this risk been included in any of the public information materials that have been provided.

It is clear that the risk of a spread or increase in *Ae. albopictus* should have been considered in a prior risk assessment as this could have serious negative implications for human health. This risk would have been included in a risk assessment that met EU standards, which is a legal requirement for the export of GM mosquito eggs from the EU.

1.2. ***Impact on target mosquito population numbers and on dengue fever***

Oxitec has not assessed the possibilities that mosquito numbers in areas neighbouring the trials could increase as a result of the experiments; a rebound in mosquito numbers or cases of disease could occur when releases cease; or partial population suppression could increase the risk of the more severe form of the disease dengue hemorrhagic fever (DHF). These possibilities are risks to public health associated with undertaking trials in dengue-endemic areas.

The EFSA Guidance includes: "*Changes in TO* [target organism] *populations caused by the GM component of the releases (size, age structure, sex ratio, fertility, mortality) that may result in adverse effects leading to environmental harm*" (page 87) and "*Loss of immunity in the human population and reliance on continued long-term positive effects of vector suppression or replacement strategy*" (page 109).

David et al. (2013) focus on malaria, but also note that: "*loss of acquired immunity may increase transmission… especially if vector suppression is only temporarily successful*".

Assessing these risks is extremely difficult due to the lack of public information. Oxitec has published the results of its population suppression trial in the Cayman Islands[20] but no results from its trials in Brazil (the only dengue-endemic country where population suppression experiments have taken place so far). In Malaysia, only a small initial trial was conducted and experiments on population suppression did not take place before the trials were terminated.

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 observed 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. A recent paper, which fits a simple computer model of mosquito populations to the Cayman Islands data, predicts that releases of 7 million GM mosquitoes a week, in an initial phase, would be needed to suppress a population of 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.[21] The authors admit that in the real world, where mosquito populations are more complicated, higher numbers might be needed. This suggests that Oxitec's technology is not very effective and the prospects for sustained suppression of large mosquito populations may be very poor.

There are only two public sources of information about the population suppression effects of GM mosquitoes in Brazil. One is a report (the PAT report) from a workshop showing 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 reported mating competitiveness was only 0.03 (3 in 100) on average and dropped to 0.012 (1.2 in 100) in the final phase.[22] 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. More recently, Oxitec has highlighted a claimed success in reducing the *Aedes aegypti* mosquito population in the village of Mandacaru in Bahia by 96%. The company has

included one graph from this experiment in a booklet on its website, but no details have been published.[23] The releases were made in a village in the dry season in order to try to improve the chances of success.

The problem with poor efficacy is not only that it is a waste of money but also that it can give rise to unnecessary risks.

The first issue to consider is whether or not releases of GM mosquitoes could cause an increase in the numbers of mosquitoes in surrounding areas. This effect is predicted by some models for the release of sterile insects.[24] Oxitec's Cayman Islands' paper and its graph from Mandacaru both show increases in *Aedes aegypti* mosquitoes in the control area, as population suppression in the target area begins. In the Cayman Islands the control area was next to the target area for the releases, but for Mandacaru there is no public information about the location of the control area. The number of mosquitoes trapped in the untreated area also increased in the final phase of the Itaberaba experiments according to the PAT report. Thus, there appears to be a real possibility that wild-type males, when swamped by very high releases of GM males, simply migrate to mate in the surrounding area, potentially increasing health risks for the people there. More information is needed to either confirm or rule out this possibility. Since Oxitec calculates population suppression based on the difference between the target area and the control area, it is possible that claims of significant drops in population partly reflect significant increases being caused elsewhere.

A second issue is whether there could be a rebound in mosquito numbers and/or cases of disease. The recently published model of Oxitec's releases in the Cayman Islands predicts a rebound in mosquito numbers when population suppression ceases. Another possibility is that there is a rebound in number of dengue cases increases due to loss of human immunity.[25,26,27] This is a possible mechanism through which the number of dengue cases could increase as a result of the experiments, especially if a reduction in the mosquito population cannot be sustained.

Perhaps the most important issue is whether cases of the more serious dengue hemorrhagic fever (DHF) might increase as a result of the experiments.

In its draft risk assessment submitted to regulators in the USA Oxitec states: "*It has been suggested that, in countries with very high transmission rates, reduction in transmission could increase the frequency of dengue hemorrhagic fever (DHF) even while decreasing the incidence of dengue fever*". The mechanism is a possible loss of cross-immunity to multiple serotypes of dengue.[28,29] Cross-immunity occurs at high frequency of biting but can reduce as the frequency of biting is reduced, leading to an increase in the frequency of DHF if the mosquito population is only partially suppressed. In its draft risk assessment for the USA, Oxitec dismisses this concern by making an unproven claim that the reduction in transmission will be well below the necessary level and pointing out that this concern is not relevant to the USA (where dengue fever is not endemic). However, this risk is highly relevant in Panama and Brazil.

A more recent paper considers both loss of cross-immunity and age-related effects ('endemic stability') for any approach that reduces frequency of biting.[30] It concludes that in areas of high mosquito abundance, mosquito control programmes should be conducted only after a vaccination programme with a high coverage has been initiated.

It is difficult to quantify this risk but it remains a matter of concern because: (i) no thresholds for dengue transmission or DHF transmission have been established in the areas of release; (ii) only limited data (no data from Brazil) have been published regarding the claimed success of Oxitec's experiments to date; (iii) dengue and DHF have not been monitored

during the Brazil experiments (and dengue is not endemic in the Cayman Islands); (iv) those results which are in the public domain suggest that the proposed releases will be inadequate to suppress the *Aedes aegypti* population sufficiently to avoid this risk.

Reportedly, the researchers involved in Oxitec's experiments in Brazil accept that there has been no reduction in dengue incidence[31], and a dengue emergency has been declared in the area where the experiments have been taking place.[32]

The risk that partial or temporary suppression of the *Aedes aegypti* population could actually make the dengue problem worse has not been considered at all in risk assessments in either Panama or Brazil. However, partial or temporary suppression of *Aedes aegypti* populations could be extremely risky in dengue endemic areas and lead to harm to public health. This risk would have been included in a risk assessment that met EU standards, which is a legal requirement for the export of GM mosquito eggs from the EU.

1.3. Release of biting females and risk of biting/ingestion of mosquitoes

One possible risk is that new proteins produced by the GM mosquitoes could have a toxic or allergic effect on humans or animals, if the GM mosquitoes are swallowed, or if female GM mosquitoes bite people or animals. Female GM mosquitoes can also spread disease. Although Oxitec intends to release only male GM mosquitoes a small proportion of females are expected to be released and some GM female larvae will also survive to adulthood.

The EFSA Guidance includes: "*Potential toxic effects of the new compound(s), their derived metabolic products and/or the GM insects to humans and animals, e.g. qualitative or quantitative change in the production of toxins by the GM insects when compared with their non-GM comparators*" and also includes "*Potential allergenic effects of the new compound(s), their derived metabolic products and/or the GM insects to humans and animals*" (page 108)

The AHTEG Guidance also includes as an issue for consideration in the ERA: "*Whether the LM* [living modified] *mosquitoes are likely to affect other organisms with which they interact (e.g., predators of mosquitoes), and whether that could lead to an adverse effect (e.g., on the food chain)*".

Reeves et al. (2012) note that: "*there is the plausible concern that females could inject tTA into humans along with mosquito salivary gland fluids that are transferred as part of a normal bite*" and that "…*tTA-expressing females would occur in the environment in at least three circumstances: firstly, if heritable resistance to the RIDL construct was to arise in the wild; secondly, while the mechanical removal of females prior to release is highly effective, it is not 100%; and thirdly, when RIDL stocks are only partially sterile under field conditions. In fact, OX513A males are only partially sterile, and when they mate with wild females they will produce 2.8%–4.2% the normal number of eggs, half of which will be biting daughters*".

Oxitec has recently published figures on the number of biting female GM mosquitoes that are inadvertently released.[33] They report that female contamination is on average 0.02%. If correct, this would mean that 200 biting female GM mosquitoes are released in every million males. Current production of Oxitec's GM mosquitoes in Brazil is 4 million a week. In the Cayman Islands, mechanical sorting was less effective, leading to about 5,000 biting female mosquitoes in every million males (additional sorting was then performed by hand).[34]

Although no official information on the scale of the releases has been provided in Panama, press reports state that the intention is to release 80,000 GM mosquitoes three times a week (240,000 a week) making a total of 5,760,000 in six months.[35] Using the figures from the sorting process in Brazil, this would mean 1152 biting female GM mosquitoes would be

released during the first six months of the experiments. Poorer sorting could release many more and additional GM females will develop from any GM larvae that survive to adulthood.

In addition to the risk of being bitten, journalists have reported that in Brazil "…*it's impossible to talk during the liberation sessions without accidentally swallowing a few*…" due to the very large numbers of GM mosquitoes being released to try to swamp the wild population.[36]

It is therefore inevitable that some people and animals will get bitten by a GM mosquito and others will swallow or consume them.

Risk assessments in Panama and Brazil have included claims that the proteins produced in the GM mosquitoes are not toxic. However, Oxitec has provided no data on the toxicity or potential allergenicity of the tTA protein expressed by its GM mosquitoes. Signs of toxicity[37] and neurotoxicity[38] have been reported in mice, yet these papers are not cited and Oxitec has provided no evidence that swallowing or being bitten by GM mosquitoes will not be harmful to humans or animals. In Spain, Oxitec has recently withdrawn an application to release GM olive flies while it undertakes further testing demanded by the regulators, including tests of toxicity to non-target species.[39] If risk assessments that met EU standards had been provided in Panama and Brazil it is clear that data on the toxicity of and allergenicity of tTA, for both humans and animals, would have been required.

1.4. **Survival and spread of GM mosquitoes and impacts of antibiotic resistance**

Oxitec's GM mosquitoes are programmed to die at the late larval stage. However, there are several mechanisms which could allow many more of the mosquitoes to survive to adulthood.

The EFSA Guidance includes: "*Reduction in efficacy of the GM insect mediated trait that may result in adverse effects*".

The AHTEG Guidance requires consideration of evolutionary effects of concern "*that could result in a breakdown in the effectiveness of the technology and the resumption of previous disease levels*".

In the laboratory, 3% of the offspring of Oxitec's GM mosquitoes survive to adulthood, even in the absence of the antidote tetracycline.[40] When GM mosquitoes were fed cat food containing industrially farmed chicken, which contains the antibiotic tetracycline, the survival rate increased to 15-18%. Oxitec originally hid this information[41] but later admitted to an 18% survival rate of larvae fed on cat food in a published paper.[42]

Oxitec claims that this survival rate will not happen in the wild because the GM larvae will breed only in clean water. However, a number of studies have found that *Aedes aegypti* mosquitoes can breed in septic tanks where there can be high levels of contamination with antibiotics such as tetracycline. [43,44,45,46,47,48] *Ae. aegypti* also commonly live in areas where discarded takeaways are likely to contain meat contaminated with tetracycline. Oxitec uses a diet supplemented with 30 µg/ml of the tetracycline to breed its mosquitoes in the lab. The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow the GM mosquitoes to breed. Oxytetracycline can be found at concentrations above 500 µg/g in animal manure and doxycycline at up to 78516.1 µg/kg dry weight in broiler manure, which is likely to be more than enough to inactivate the killing mechanism.[49,50]

The issue of GM mosquitoes breeding in areas contaminated with tetracycline has not been considered in risk assessments in either Panama or Brazil.

The use of tetracycline to breed the GM mosquitoes in the lab also carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health. Insect guts are reservoirs for antibiotic resistance genes with potential for dissemination.[51,52] Insect production in factories exposed to antibiotics could lead to drug resistance in their microbiota so that the insects disseminate antibiotic resistance when released into the environment.[53,54] This issue has not been considered in risk assessments in either Panama or Brazil.

The percentage of surviving GM mosquitoes could also increase if resistance to the genetic killing mechanism evolves over time. [55] This issue has also not been considered in risk assessments in either Panama or Brazil.

Increased survival rates would reduce the effectiveness of any population suppression effect over time, increase the number of biting GM females, and potentially allow the GM mosquitoes to establish in the wild. The potential spread of antibiotic resistance could pose a serious risk to human and animal health. These risks therefore need to be considered in the risk assessments. This would have been the case had risk assessments which met EU standards been provided by the exporter.

1.5. Transfer of other traits to wild mosquitoes

Oxitec's GM mosquitoes have been developed from a non-native strain. In the Cayman Islands, the OX513A insertion in *Aedes aegypti* (originally developed from a Rockefeller strain[56]) was introgressed into a Mexico-derived genetic background by five generations of back-crossing;[57] it appears that this same strain was then used in Brazil and probably also in Panama. Oxitec has not published any information about the origins of the Mexican strain and it does not appear to have tested the back-crossed strain for insecticide-resistance or disease transmission properties.

When Oxitec's GM mosquitoes breed with wild mosquitoes some of their other genetic characteristics will be passed on to the local wild mosquito population. Different strains of the same species are found in different places and some strains are more resistant to insecticides than others or better transmitters of disease (the four serotypes of the dengue virus and/or other viruses, such as Yellow Fever). The possible introduction of such traits needs to be considered. Harm to people's health can be increased if some serotypes or viruses can be transmitted more easily by the introduced strain than they were by the wild species already in the area, or if the strain is resistant to insecticides. These risks have not been included in the risk assessments in Panama or Brazil.

For comparison, in the UK, Oxitec has been prevented from releasing a GM diamondback 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.[58] It is therefore clear that if the risk assessments had met EU standards, this risk would have been considered.

Conclusion

People in Brazil and Panama have been subjected to unnecessary risks to human health and the environment as a result of the failure of the UK company Oxitec to follow the transboundary notification process correctly and provide an environmental risk assessment that meets EU standards. Both exporting and importing Parties (the UK, Panama and Brazil) have failed to ensure that risk assessments are provided by the exporter or meet the necessary standards.

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