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Safety of transgenic *Aedes* in Brazil

This online first version has been peer-reviewed, accepted and edited,
but not formatted and finalized with corrections from authors and proofreaders.

Use of transgenic *Aedes aegypti* in Brazil: risk perception and assessment

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(Submitted: 10 March 2016 – Revised version received: 16 June 2016 – Accepted: 22 June 2016 – Published online: 31 August 2016)

Abstract

The OX513A strain of *Aedes aegypti*, which was developed by the British company Oxitec, expresses a self-limiting transgene that prevents larvae from developing to adulthood. In April 2014, the Brazilian National Technical Commission on Biosafety completed a risk assessment of OX513A and concluded that the strain did not present new biological risks to humans or the environment and could be released in Brazil. At that point, Brazil became the first country to approve the unconstrained release of a genetically modified mosquito. During the assessment, the commission produced a comprehensive list of – and systematically analysed – the perceived hazards. Such hazards included the potential survival to adulthood of immature stages carrying the transgene – should the transgene fail to be expressed or be turned off by exposure to sufficient environmental tetracycline. Other perceived hazards included the potential allergenicity and/or toxicity of the proteins expressed by the gene, the potential for gene flow or increased transmission of human pathogens and the occupation of vacant breeding sites by other vector species. The Zika epidemic both elevated the perceived importance of *Ae. aegypti* as a vector – among policy-makers and regulators as well as the general public – and increased concerns over the release of males of the OX513A strain. We have therefore reassessed the potential hazards. We found that release of the transgenic mosquitoes would still be both safe and of great potential value in the control of diseases spread by *Ae. aegypti*, such as the chikungunya, dengue and Zika virus disease.

Introduction

In April 2014, Brazil's National Technical Commission on Biosafety – i.e. the agency officially responsible for the assessment of the risks posed by genetically modified organisms in Brazil – assessed the potential risks of the release in Brazil of the transgenic OX513A strain of *Aedes aegypti* and concluded that such a release would be safe.¹ At that point, Brazil became the first country to approve the unconstrained release of a genetically modified mosquito. Two years later, however, the Zika epidemic had added to the general public's concerns over the release of mosquitoes and we therefore decided to re-investigate the perceived risks and update the commission's risk assessment.

Control of arbovirus vectors

Several arboviruses – e.g. chikungunya virus, dengue viruses and, more recently, Zika virus – cause much human suffering in Brazil.^{2,3} In 2015, for example, there were more than 1.5 million suspected cases of dengue fever in the country.² While attempts to create effective or cost-effective vaccines continue, control of the mosquito vectors remains of the utmost importance.⁴ Brazil has had some success in controlling mosquitoes, even early in the 20th century, when no effective insecticides were available.⁵ Mosquito control – by residents, health workers and other municipal workers supported by a heterogeneous and broad set of collaborators – remains the focus of the National Dengue Control Programme.⁶ However, the failure of this programme to reduce vector populations to levels that could interrupt dengue transmission⁷ has spurred Brazilian interest in dengue vaccines⁸ and novel approaches to vector control such as the sterile insect technique.^{9–13} The International Atomic Energy Agency describes the sterile insect technique as “a type of birth control in which wild female insects of the pest population do not reproduce when they are inseminated by released, radiation-sterilized males”.¹⁴ Although sequential releases of large numbers of the sterilized males should lead to a reduction in the size of the pest population, the irradiation used can reduce the released insects' competitiveness – and this appears to be a particular problem when the insects involved are mosquitoes.¹⁵ Over the last decade, as an alternative to the sterile male technique, the genetic modification of mosquitoes, with the production of large numbers of males and females that carry a self-limiting transgene, has been investigated.¹⁶ Once released in the environment, the male insects carrying the transgene – which have to be produced in the presence of a selective agent that blocks the transgene's expression – should, potentially, compete on equal terms with the wild males.^{17–20}

The OX513A strain of *Ae. aegypti*, which was developed by the British company Oxitec, expresses a self-limiting dominant transgene that is able to kill, at larval stage, the mosquitoes in which it is expressed (Fig. 1).²¹ In the presence of tetracycline, the transgene is either not expressed or is only expressed at a very low and non-lethal level. Once released in the environment, most males carrying the transgene die after about two days.²¹

Risk perceptions and assessments

In almost all countries, any living modified organism derived from modern biotechnology is very strictly regulated and subjected to a long and detailed risk assessment.²² In Brazil, which is a Party to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, such risk assessment is the sole responsibility of National Technical Commission on Biosafety.²³ The commission consists of 27 members and an equal number of surrogates – chosen by various federal government agencies and civil organizations – including highly qualified scientists from across the country. As stipulated in the Cartagena Protocol, any risk assessment of a genetically modified product has to be supported with so-called hard data and preferably with hard data collected in the country that intends to use the product. In 2011, the commission was asked to conduct a risk assessment of the biosafety of the OX513A strain of *Ae. aegypti* as a precursor to the experimental, small-scale release of adult males of the strain – first in Juazeiro and then in Jacobina – in Brazil's Bahia state in 2013. The commission used data collected in these releases and in field experiments conducted in the Cayman Islands and Malaysia, along with some relevant laboratory results, to produce a comprehensive risk assessment.¹ In April 2014, on the basis of this assessment, the commission approved the OX513A strain for unconstrained release throughout Brazil, primarily as a method of dengue control.

Risk assessments by the commission, like those by the majority of the agencies tasked with assessing the risks posed by the release of a genetically modified organism, have four main steps that comply with the Cartagena Protocol: (i) problem formulation; (ii) risk characterization; (iii) risk classification; and (iv) an overall safety evaluation.^{24,25} In problem formulation, specialists outline the release scenario and list all the associated hazards – as perceived by themselves, by other specialists and by the general public. Each perceived hazard is then assessed, in the context of the planned release, using any relevant data that are available and considering each plausible route by which the hazard may cause harm. In this risk characterization, any perceived hazards that cannot plausibly lead to harm are excluded. In the subsequent risk classification, the risk of each of the remaining perceived hazards

occurring is estimated. Finally, the assessors estimate the overall risk posed by the planned release and consider whether the organism should be considered safe for the environment.

Although international guidelines on the assessment of food safety²⁶ do not apply directly to mosquitoes, they can still be useful in assessing the allergenicity and toxicity of novel proteins created as the result of the genetic modification of such insects.

2014 assessment

As part of its risk assessments of the OX513A strain in 2014, the Technical Commission listed the potential hazards. Although some of the listed hazards were perceived by specialists, most had been voiced by the general public. Among the main perceived hazards were the unexpected survival of at least some of mosquitoes that carried the transgene and/or their progeny as the result of partial penetrance and/or the presence of tetracycline in the environment, allergenicity and/or toxicity of two new proteins expressed, possibly in the mosquitoes' saliva, as a result of the genetic modification, the vertical and horizontal flow of the transgene and its consequences, the participation of transgenic mosquitoes in the transmission of dengue viruses and the occupation, by other vector species, of breeding sites made vacant by the intervention.¹

Only one of the listed perceived hazards – i.e. survival in the presence of tetracycline – was considered plausible and even that hazard was deemed unlikely since concentrations of tetracycline found in freshwater are usually more than two orders of magnitude lower than that needed to block the transgene's expression in laboratory-bred insects.¹ In addition, only 50% of the progeny of any female mosquitoes carrying the transgene into adulthood would carry the lethal gene – and die at the larval stage if not also protected by exceptionally high concentrations of tetracycline.

Although the occupation of breeding sites, cleared of *Ae. aegypti*, by other vector species – e.g. *Ae. albopictus* – was deemed a negligible risk by the entomologists involved in the risk assessment, it remained an area of concern among a few of the commission's non-specialists.

The commission approved the nationwide release of adult males of the OX513A strain on the condition that Oxitec investigated tetracycline concentrations in potential breeding sites – by means of periodic literature reviews – and also investigated the occupation of cleared breeding sites by *Ae. albopictus*.

2016 re-assessment

By 2016, the recent Zika outbreak had led to additional concerns about the release of any *Ae aegypti* in Brazil, although few of these concerns had been voiced by universities, research centres or official risk assessment agencies. Such new concerns had arisen even though, by 2016, the usefulness of genetic modification in the control of mosquitoes had been demonstrated in Brazil and elsewhere^{17,19,27} and there appeared to be ever-growing acceptance of such an approach to vector control – among the general public and public health managers. Among the new concerns were the potential participation of released mosquitoes, or their progeny, in the transmission of Zika virus and the perceived possibility of horizontal gene transfer from the transgenic mosquitoes to the Zika virus – potentially making the virus more harmful to humans. Although, to us, neither of these newly perceived adverse effects of the release of transgenic mosquitoes in Brazil appeared plausible, in April 2016 we re-assessed the general safety of the unconstrained release of males of the OX513A strain of *Ae aegypti* in Brazil.

The plans for the commercial releases of males of the OX513A strain throughout Brazil have not been changed since the 2014 risk assessment. It is expected that the releases will take place mainly in cities or other densely populated areas, in association with other control measures and under continuous surveillance by the relevant municipal health authorities and Oxitec. At the time of writing, a large-scale but still pre-commercial release is in progress in Piracicaba county in São Paulo state. The data already collected during this release (Fig. 2) confirm the effectiveness of such releases, support the encouraging results previously obtained in the Cayman Islands, Malaysia and the Brazilian town of Juazeiro,^{19,27} and support the commission's earlier conclusion that such releases are safe.

As male mosquitoes do not feed on blood, the intention is to release only male *Ae. aegypti*. However, there is a small margin of error during the separation of males and females before field release and, in consequence, up to 0.2% of the released insects may be females.²⁰ Although the bites of the released females are clearly perceived as a risk by the general public, they are relatively rare and most of the released females will be dead within two days – i.e. too soon for them to become infectious even if they have taken a blood-meal that contains chikungunya virus, dengue virus or Zika virus.²⁹ Even if, as may occasionally happen, a female carrying the transgene survives long enough to become infectious, there is no evidence that it will be any worse as a vector than a wild *Ae. aegypti*.³⁰

As genomic analyses have revealed many examples of gene transfer between distinct taxa,³¹ horizontal gene transfer is always a controversial issue in discussions about the unanticipated risks of releasing a genetically modified organism. However, such gene transfers appear to be very infrequent. There is evidence of horizontal gene transfers from invertebrates to mammals but all such transfers that have been detected appear to be transposon-dependent and very infrequent.^{32–34} It does not seem possible that the piggyBac transposon used in the construction of the OX513A strain of *Ae. aegypti* could mediate the transfer of genetic information from the mosquito to other genomes. This transposon does not codify transposase and, since transposase is not codified in the mosquito's genome, the insert cannot be remobilized.

Even if a transposase were present, there seems to be no risk of genetic transfer between the OX513A strain's transgene and the Zika virus because the viral genome is made of single-stranded ribonucleic acid, which is not a substrate for transposases. When, in an effort to understand the Zika outbreak in Brazil, various isolates of the Zika virus were sequenced, no evidence was found for transposon insertion or any other form of horizontal gene transfer.³⁵

Conclusion

After circulating around the world, the Zika virus is rapidly spreading through the Americas.³⁶ The detection of an association between microcephaly in a baby and maternal infection with Zika virus^{37–41} has greatly increased the perceived importance of the primary vector of Zika virus – i.e. *Ae. aegypti* – among both the general public and policy-makers.⁴² The results of our re-assessment of the safety of releasing males of the genetically modified OX513A strain of *Ae. aegypti* – as well as the results of a related assessment by the United States Food and Drug Administration⁴³ – indicate that releases of such mosquitoes still offer a safe and potentially effective way of reducing wild populations of the vector. The risk-assessment components adopted by both the commission and the United States Food and Drug Administration⁴³ are those recommended by the World Health Organization.⁴⁴ In the ongoing battle against the Zika outbreak, the Brazilian health managers' choice of control methods has to be based on careful risk assessments and not on risk perceptions. Public perceptions – especially with the advent of fast and global communications via the Internet – are often inaccurate and the general public is often misled by conspiracy theories and catastrophism.^{45–}
⁴⁷ In the field of mosquito control by genetic modification, Brazil is a world leader. Brazil has already evaluated the OX513A strain of *Ae aegypti* and approved the strain's commercial use.

Once the strain has been registered at Brazil's National Health Surveillance Agency, it will probably be commercially released throughout the country.

Acknowledgements

We thank Dr Christie Wilcox.

Competing interests:

None declared.

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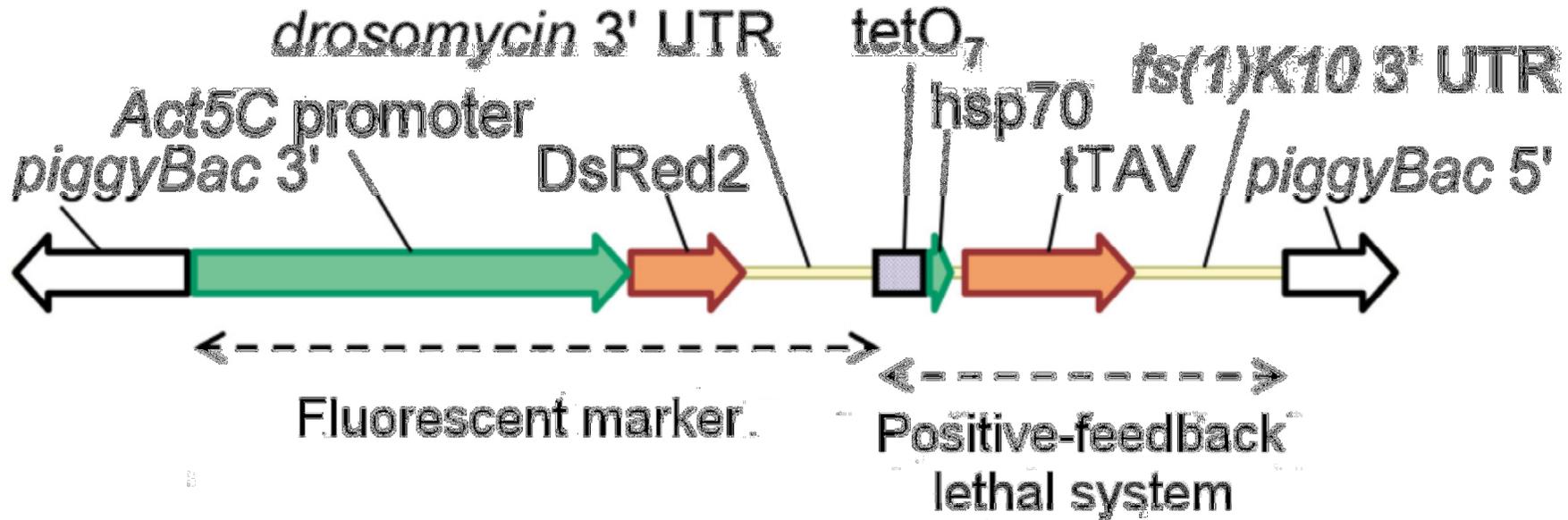
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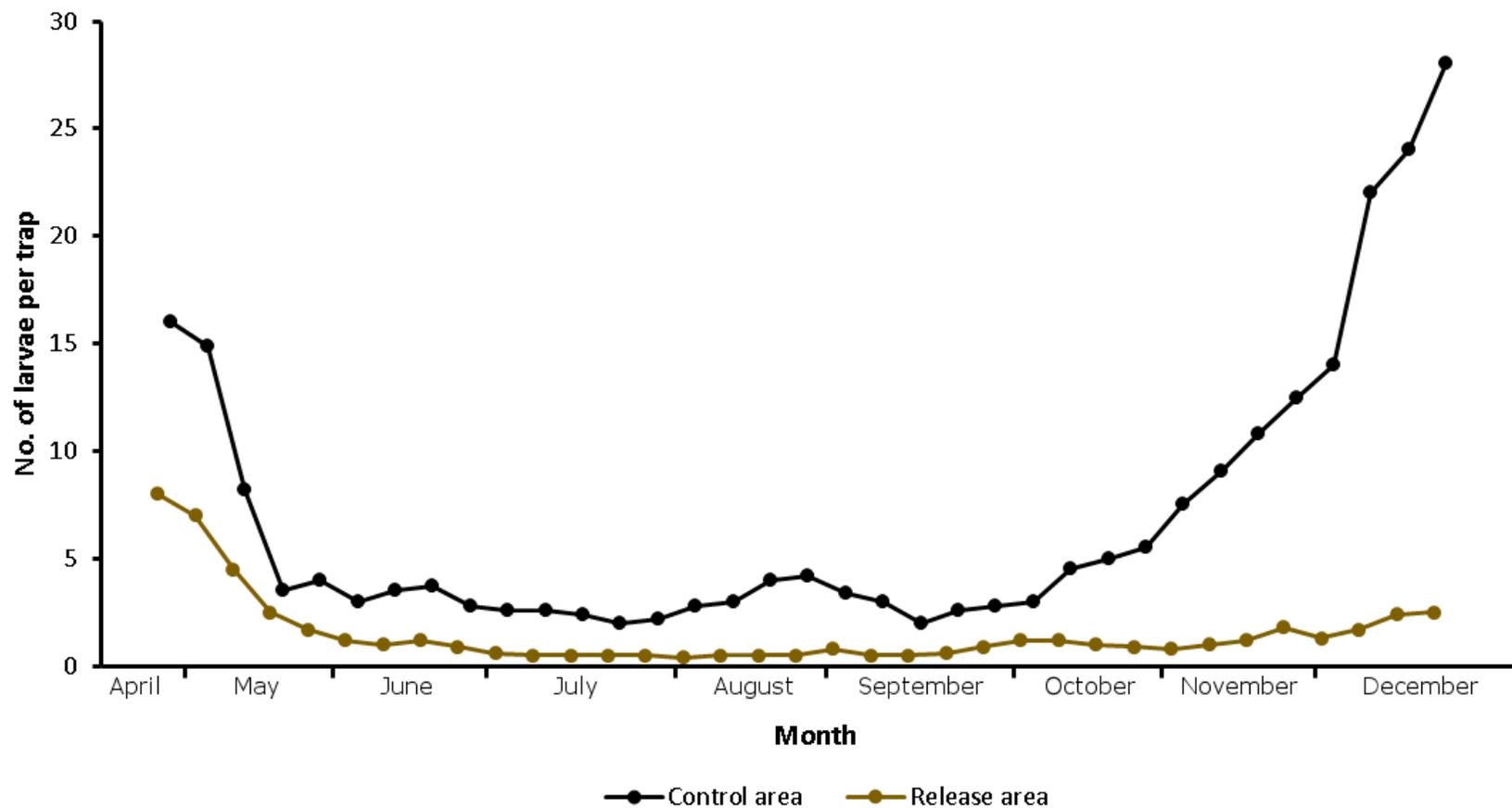
Fig. 1. Structure and function of the OX513 insert, a transgenic construction inserted into the *Aedes aegypti* genome



tTAV: tetracycline-repressible transcriptional activator; UTR: untranslated region.

Notes: The insert is a non-autonomous transposon derived from a piggyBac transposon. Transformants may be traced via the fluorescent protein expressed by the DsRed2 gene, which expression is driven by the Act5C promoter. The tetracycline-repressible transcriptional activator is under control of its own binding site, tetO₇ – a minimal *Drosophila* hsp70 promoter. The increase in the intracellular concentration of this activator disrupts cell metabolism and leads to cell death. Although the insert's 5' and 3' ends allow the insert's insertion in double-stranded deoxyribonucleic acid in the presence of a specific transposase, the insert cannot be further mobilized because the necessary enzyme is not expressed by the transgenic mosquito. Drosomycin 3' UTR and fs(1)K103' UTR are spacers.²¹

Fig. 2. Numbers of mosquito larvae in traps set up in a control area and in an area where males of the transgenic OX513A strain of *Aedes aegypti* were released, Piracicaba county, Brazil, 2015



Notes: The transgenic male mosquitoes were released throughout 2015. We have permission to republish the figure from the British company Oxitec. The data shown were previously presented in a blog.²⁸