GeneWatch UK comments on Risk Assessment report of the Malaysian Genetic Modification Advisory Committee (GMAC) for an application to conduct a limited Mark-Release-Recapture of Aedes aegypti (L.) wild type and OX513A strains[1]

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Introduction

GeneWatch UK is a science-based not-for-profit organisation, which aims to ensure that genetics is used in the public interest. GeneWatch supports a precautionary approach to the release of genetically-modified organisms (GMOs) into the environment, in line with international conventions. Our comments on the GMAC's Risk Assessment report are intended to assist the Committee in ensuring that the relevant scientific evidence, unknowns and uncertainties are considered fully in the risk assessment. We recognise that the release of genetically modified (GM) insects poses new challenges which test the limits of the expertise available in academic institutions and NGOs worldwide. Our comments are therefore not a criticism of the committee, members of which are experts in their own right, but are a constructive attempt to raise important issues that may not have been considered previously.

GeneWatch UK has conducted and published an investigation of the UK-based company Oxitec's role in the development, patenting and promotion of the use of genetically-modified (GM) mosquitoes.[2] We are concerned that the novelty of this application of GM technology has made regulators in several countries too dependent on advice provided by the company, which has a vested interest in speeding its products into the market place in order to generate financial returns for its investors. In GeneWatch's view this means that a number of potential risks have been omitted or downplayed.

In making these comments we wish to emphasise that our own review of the relevant literature is still incomplete and that many of the questions that we raise appear to be unanswerable in the current state of knowledge. Whilst it is possible that future field trials might fill some of these gaps, our view is that such trials are premature at the current time. This is because other missing knowledge, which could be obtained only by studying wild-type mosquito populations and the dengue virus in more depth and by additional laboratory experiments, is a prerequisite to understanding the impacts of open releases of GM mosquitoes on biodiversity and public health.

Overview of risk assessment process

The GMAC has followed a risk assessment process in which it has:

1. Identified potential hazards;
2. Evaluated the likelihood of hazards;
3. Evaluated the consequences of each hazard;
4. Calculated overall risk.

This is a systematic approach which has much to recommend it. However, the Risk Assessment (RA) process would have been more transparent had the GMAC also listed all the potential hazards it has identified and its evaluations of their likelihood,

consequences and estimated overall risk. A description of the process the GMAC has used to undertake these steps (i.e. how it has made its evaluations) would have also increased transparency, which is an important factor in decision-making, particularly given the intense interest and debate over this new area of transgenic technology.

The lack of clarity appears to be due largely to the novelty of the application, which requires the risk assessment of an entirely new application of transgenic technology. This introduces a number of important issues, some of which are familiar in other contexts but none of which have previously been applied to GM insects, especially to insects which are a known vector of human disease.

The result is that, in our view, the risk assessment process is incomplete, due to the lack of:

1. a full literature review;
2. consideration of the important role of ecological and disease transmission modelling in the risk assessment process;
3. a formal, systematic approach to expert elicitation;
4. a step-by-step approach;
5. full appreciation of the novelty of the proposal and the importance of scientific unknowns and uncertainties;
6. a process to identify information needs that are relevant to the ultimate policy decision (i.e. whether or not to deploy this technology on a commercial scale).

Each of these issues is considered in turn below.

Literature review

The GMAC has cited a number of scientific journal publications and reports and lists 27 references.

However, this is an incomplete review of the relevant literature in this field. For example, a report to the European Food Safety Agency on developing a process for risk assessment of GM insects lists more than three hundred references.[3] The Mosqguide project (in which Oxitec is a partner) lists 185 references[4] and Oxitec lists 32 of its own publications on its website.[5] There are also several books[6] published in this field. Numerous other relevant papers exist in the scientific literature, for example, on the epidemiology of the dengue virus.[7,8] Theses[9,10] are also available on relevant topics and may contain some relevant missing information in this new and emerging field.

Without a comprehensive literature review, hazards may be missed or their importance underestimated. For example, the effects of GM mosquito releases on the evolution of the dengue virus[11] and on human immunity[12] are considered important in the scientific literature, but are not listed in the bibliography provided. It is therefore unclear whether these effects were considered by the GMAC in reaching its conclusions.

Role of ecological and disease transmission modelling

Ecological and disease transmission modelling plays an important role in risk assessment because without such modelling it is impossible to predict the complex interactions between predators and prey, GM and wild-type mosquitoes, and effects such as co-evolution of the dengue virus.

Oxitec has developed a number of ecological models to attempt to predict the consequences of releasing GM mosquitoes on the wild-type population.[13,14] It is surprising that these papers are not cited in the bibliography since their conclusions and limitations provide important information about potential adverse effects and whether or not the likelihood of these can be determined.

In the most recent of these papers[15], Oxitec scientists find that the traditional sterile insect technique (SIT) can increase mosquito populations in the targeted and surrounding areas, and also result in large fluctuations in populations. Oxitec concludes that its own GM technique will not cause these adverse effects due to the additional effect on population suppression of competition for food between the GM and wild-type larvae. However, this conclusion is at best preliminary because, despite the improved sophistication of this model, it remains highly simplistic compared to the real-world situation. For example, the model includes only one species of mosquito, although there are serious concerns that the Asian Tiger mosquito *Aedes albopictus* may move into the ecological niche vacated by falling populations of *Aedes aegypi* (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[16]). Complex interactions between competing mosquito species, their predators and environmental factors such as climate have been identified in the literature but have yet to be included in ecological models.[17,18,19] Populations can also fluctuate significantly and the combined effect of insecticide use and GM mosquito releases needs to be considered.[20] Further, Oxitec's model has not been validated i.e. it has not been compared with data and shown to accurately reproduce complicated real-world ecosystem responses. This issue is considered further below (see 'step-by-step approach').

Even if the effect of the releases on mosquito populations is better understood, the potential effects on dengue transmission may not be beneficial. Oxitec's own assessment states[21]: "*The consequences on the epidemiology of dengue fever might necessitate further theoretical assessment involving the disease's transmission dynamics as well as the population dynamics of the vector*". However, no models of disease transmission appear to have been published by Oxitec or cited in the Risk Assessment. This is problematic because several authors have warned that a reduction in human immunity combined with residual disease transmission (from *A. aegypti* or *A. 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.[22,23] Research is ongoing to attempt to establish the relationship between *Aedes aegypti* population densities and dengue transmission.[24] However establishing disease transmission thresholds remains a long-term goal, although it is considered a prerequisite to understanding the impact of vector interventions (including the release of GM mosquitoes). Indeed it has been described by one author as "*among the most important unanswered questions in dengue epidemiology and GMM* [Genetically Modified Mosquito]*-based control approaches*"[25]

In addition, no models of potential evolution of the dengue virus appear to have been published by Oxitec or cited in the Risk Assessment. The possibility that the dengue virus may evolve to become more virulent is considered a lower risk with population suppression approaches, such as Oxitec's, than with other GM approaches[26]. However, such effects are still at an early stage of study and will be complicated by the need to include all four serotypes of the dengue virus in future disease transmission models.

Modelling is also critical to establish the 'release ratios' needed to maintain populations below the threshold for disease transmission: particularly because a reduction in disease transmission that is not sustained can be disastrous due to the expected reduction in immunity,[27] but also because this will impact on ongoing costs and cost-effectiveness of any sustained commercial releases that may take place in the future. Current estimates of release ratios vary widely and disease transmission thresholds have yet to be considered. [28,29] However, weekly releases of millions of mosquitoes are expected to be needed, with some researchers suggesting that males would need to be released every 50m along urban streets to find all the local females. [30,31]

Although small-scale experimental releases will not be as risky as full-scale commercial releases it is important to have an a priori understanding of the current limitations of ecological and disease transmission models in order to (i) improve the expert elicitation process; (ii) take a step-by-step approach to risk assessment so that decisions on both experimental and commercial releases are fully informed; (iii) fully inform local people of the purpose of the trials, in order to meet the requirements for consent. These issues are discussed further below.

Expert elicitation

The GMAC appears to have used a process of expert elicitation, similar to that described in the report of the NRE-UNDP-GEF workshop on Risk Assessment of Transgenic Insects in Malaysia in November 2008 to make its qualitative evaluations and to rank hazards.[32] It has used its own expertise, plus discussions with the company and one NGO to do this.

This process has allowed the GMAC to identify two important hazards (possibility of unintentional release of females; and introduction of the transgene into the wild population due to reduced gene penetrance). GMAC has identified the need for management strategies to attempt to limit any impact of these hazards.

The GMAC has rightly sought the views of experts in the company and NGOs and included the issues raised in the risk assessment process. However, the lack of a formal expert elicitation process, informed by a prior literature review and more sophisticated ecological and disease modeling, has limited the value of this process.

Expert elicitation is a recognised method of obtaining inputs from experts to risk assessment processes. It is normally used to help to identify and address uncertainties. It is most often used to quantify ranges for poorly known parameters, but may also be useful to further develop qualitative issues such as definitions, assumptions or conceptual (causal) models.[33] Thus it does not replace the use of ecological or disease transmission modeling but may help to define parameters or highlight effects that should be included in the models.

In addition, the elicitation of information from experts hinges on the availability of expertise in the scientific community. Experts cannot make up knowledge that does not exist yet in one form or another. However, when issues are highly uncertain, controversial, unquantifiable or associated with potentially irreversible damage; or when decision stakes are very high, there may be insufficient expertise available to derive any valid judgments.[34]

Given the novelty of the product that is being assessed, there will rightly be doubts, despite the best efforts of the GMAC members, about whether the expertise to identify and assess hazards in this situation is currently available. In this situation, the lack of transparency about how GMAC reached its decisions on risk characterization (particularly the ranking of hazards and their likelihood and consequences) is of significant concern.

Further, the GMAC seems to be too heavily dependent on input from the company and from NGO experts who have limited resources (and which in the case of WWF, has stated that it lacks the expertise to comment on this new technology). Expert elicitation requires the input of a wide range of views. It is unclear whether the GMAC ensured the inclusion of critical experts, as well as advocates, of this technology: for example, by contacting experts in India who have decided not to move to open release trials of Oxitec's mosquitoes[35] and/or by involving experts in disease transmission and other aspects that have not been addressed by the company.

Importance of a step-by-step approach and understanding the role of the experiments

It is questionable whether a move to open field trials is justifiable without taking a step-by-step approach.

The GMAC has rightly noted the importance of certain risks and baseline data being understood more thoroughly prior to any larger scale or commercial release.

However, a step-by-step approach would require characterization of all the processes and parameters that need to be understood in order to ascertain whether future commercial releases of the product would lead to overall benefit or harm to health and to biological diversity. A step-by-step approach would also lead to clarity about the role of the experiments (i.e. why they are being done) and about whether or not they can contribute to significantly increasing confidence in any future risk assessment prior to a large-scale release.

A step-by-step approach would require as a first step (i.e. prior to any open release) the development of computer models that include both of the two dengue-transmitting mosquito species, their predators and prey, all four serotypes of the dengue virus, other diseases known to be transmitted by these species, and the impacts of these viruses on human health (including the role of infection by more than one virus and the relevant interactions with immunity and severity of disease).

These models would, in the first instance, not be realistic. This is due to unknown or uncertain input parameters and significant gaps in existing knowledge (particularly regarding interactions between species and the evolution of viruses and herd immunity). However, the development of such models would allow these gaps to be identified and relevant field studies and experiments devised to refine and ultimately validate the models (i.e. to demonstrate their ability to predict mosquito population fluctuations and outbreaks of dengue and other diseases in the field). The initial objective should be to predict the unperturbed system sufficiently well to give confidence that the consequences of introducing a genetically-modified species might also be predicted with a high degree of confidence. Once this objective has been achieved, sensitivity analyses

could be performed with models including the effect of the conditional lethality trait and the key unknowns and uncertainties could again be identified for further investigation. Ultimately, all uncertainties and unknowns cannot be removed or identified but the process of model validation should give confidence that risk predictions are 'fit for purpose' and that members of the public can have a high degree of confidence in the final assessment. Monitoring and follow-up would of course also be needed following any releases to check that the real world is behaving as predicted.

This process has been initiated by Oxitec in developing its existing models, but currently is far too simplistic (including only a single species and omitting disease transmission, for example) to give confidence that the model is reliable. In addition, computer models of complex systems require validation with experimental data before they can be accepted as having any predictive value.

The complexity of the relevant systems and their interactions should not be underestimated. For example, a new assessment indicates that preserving intact ecosystems and their endemic biodiversity should generally reduce the prevalence of infectious diseases, but many uncertainties remain.[36]

At the same time as developing more sophisticated ecological models to underpin the risk assessment process, laboratory experiments could address other uncertainties such as the stability of the strain over more than sixty generations. The reasons for the unexpected reduced penetrance of the conditional lethality trait also need to be better understood, as this could be an early sign of future failure of this technology. Other issues that clearly merit further investigation in the laboratory include Oxitec's misleading claim that the 3-4% survival rate of the progeny of the GM mosquitoes is of no importance because this is a low fitness genotype. In fact, there is evidence in the literature that low fitness genotypes can persist for many generations[37] and it is also possible that low fitness genotypes might also act as stepping stones for future evolution.[38] It is worth noting that some authors have suggested that adding an additional lethality factor to ensure complete penetrance "*will be necessary to avoid an unwanted ingression of adult transgenic individuals into wild mosquito populations that would interfere with monitoring of the SIT programme and could become the basis for resistance development*".[39] Experience from past Sterile Insect Technique (SIT) progammes suggests that resistance to mating with sterilized males can evolve and limit the long-term efficacy of such programmes (although it should be noted that it is not in fact correct to describe Oxitec's RIDL insects as sterile).[40]

More consideration also needs to be given to the impacts of the large numbers of dead and rotting larvae and pupae which will result from the expected death of 96-97% of the GM progeny. Are predators expected to increase due to feeding on these dead larvae and pupae (if so, which species and what are their effects)? Could there be any adverse effects on ecosystems or on drinking water? The dead and surviving GM larvae and pupae will include some infected with the dengue virus by transovarial transmission (i.e. from the maternal body into the eggs in the ovary) from their wild-type female parents.[41] The transovarial transmission of the dengue virus in both the *Aedes* vectors needs to be considered as studies in Malaysia have highlighted that both *A. aegypti* and *A. albopictus* larvae may become a reservoir of the virus during inter-epidemic periods.[42]

Finally, yellow fever has never been documented in Asia and one possible contributory factor is human immunity to yellow fever due to the presence of dengue virus antibodies

(a protective effect was found in some experiments in monkeys in the 1970s).[43] Therefore, the possibility that a reduction in human immunity to dengue could (in combination with other factors) lead to the establishment of yellow fever in Malaysia also needs to be investigated.

The lack of a step-by-step approach raises doubts about the justification for the proposed open release experiments, i.e. to compare and evaluate the longevity, dispersal distance, morphology and life history traits of the OX513A(My1) mosquito. Whilst this would undoubtedly be important information to obtain before proceeding to a large-scale commercial release, such information is of secondary importance compared to understanding the 'baseline' of how the existing wild-type mosquitoes interact with other species, viruses and humans. The very limited existing understanding of the relevant natural systems and of the stability and characteristics of the GM trait suggests that open release experiments are premature.

Novelty of the proposal and its role as a medical experiment

The GMAC cites the US Department of Agriculture–Agriculture Pest & Health Inspection Service (USDA APHIS) 2008 Environmental Impact Assessment (EIA) regarding a GM pink bollworm strain similar to that used in the GM mosquito in the application. However, as far as GeneWatch is aware, GM bollworms containing the conditional lethality trait have not been released in the United States. Only bollworms containing the fluorescent trait have been released and these have been sterilised using irradiation (as described on Oxitec's own website[44]): this means that unlike Oxitec's products they do not reproduce. Trials using sterile bollworms have been reported in the scientific literature: but these are not transgenic.[45]

Thus, the US EIA must be regarded as somewhat speculative since it has not been confirmed by experimental data. Further, previous assessments of GM technologies by USDA APHIS have not always been considered adequate by the US courts[46] and the US is not a party to the Cartegena Protocol.

In any case, and perhaps more importantly, trials of GM agricultural pests differ substantially from trials of GM mosquitoes in two respects. Firstly, populations of agricultural pests are widely regarded to be limited largely by their ability to breed and not by food supplies and competition for resources (known as 'density dependent' effects): this makes ecological modelling of population responses to the release of sterile (and perhaps also RIDL) insects simpler than it is for mosquitoes (for which 'density dependent' effects are known to be important).[47] Secondly, agricultural pests are generally not vectors of human disease, rendering modelling of disease transmission and complicated interactions between species, viruses and humans unnecessary in the case of agricultural pests. Use of GM mosquitoes in an attempt to reduce dengue transmission is thus entirely novel in that it is a medical experiment, potentially leading to a large-scale public health intervention in the future (i.e. large-scale commercial releases). In this sense, the proposed releases are radically different to any previous deliberate release of a GM organism in the policy and regulatory issues that they raise, as well as in the novelty of the technology.

Thus, for example, in the United States, USDA APHIS has no explicit authority to consider public health. The Public Health Service, the Food and Drug Administration (FDA) and the National Institutes of Health (NIH) would therefore also all likely play a

role in any future assessment were open releases of GM mosquitoes to be proposed there.[48]

Releases of insects sterilised by irradiation or mutagenic chemicals (the sterilised insect technique, SIT) were quite widely investigated in the 1970s (mainly for agricultural pests), but with some exceptions (such as the eradication of screwworm fly in Florida in 1958 using 2 billion irradiated insects, at a cost of about US$10 million[49]) these applications have rarely been successful.[50] Experiments with SIT mosquitoes in India were terminated amid controversy in the 1970s, although there have been more recent attempts to revive the idea.[51,52,53,54] However, modelling by Oxitec and others suggests that the use of the traditional SIT for mosquitoes could do more harm than good because it may in practice increase populations (due to 'density dependent' effects that are not expected when the SIT is used on agricultural pests).[55] There are no current SIT programmes involving mosquitoes or other human disease vectors anywhere in the world.

Oxitec has exported GM mosquito eggs to India for use in contained trials. However, the use of a deal with a private lab and the absence of specific guidelines for regulatory assessment of GM insects have raised concerns at the Indian Council of Medical Research.[56] To date, no application for open field trials has been made to the Genetic Engineering Appraisal Committee in India.

Open releases of GM mosquitoes have already taken place in a British Overseas Territory (Grand Cayman). However, the Cayman Islands are not covered by the Cartegena Protocol and the process of approval did not include public consultation or a consent procedure. This lack of consent or published risk assessment for the Cayman trials has attracted strong criticism from both scientists and NGOs.[57,58] Although a reduction in the *Aedes aegypti* population has been reported in the press as a result of these experiments, no data on dengue transmission will have been obtained because dengue is not endemic to the Cayman Islands. *Aedes albopictus* has been collected sporadically from a number of locations in Georgetown but has not been observed elsewhere in Grand Cayman.[59] Thus, data from Grand Cayman can also shed little light on concerns about the likely impact of releases of Oxitec's GM mosquitoes on *Aedes albopictus* populations in Malaysia (where this is an abundant native species).

GMAC has rightly considered the proposed mosquito releases under the Biosafety Law, which has been developed to implement the Cartegena Protocol to the Convention on Biological Diversity (CBD), to which Malaysia is a party. However, proper application of the Biosafety Law is necessary but not sufficient. The objectives of the CBD are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. Thus, whilst risks to human health posed by living GMOs (LMOs) are taken into account in risk assessments conducted under the CBD, assessing the pros and cons of public health interventions is not central to its purpose. Experimental releases of GM mosquitoes should also be considered as a medical experiment: hence requirements which fall outside the CBD also apply.

The GMAC has therefore rightly made prior consensus and approval from the inhabitants a condition in the release sites. 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.[60] Consent to medical experiments

is the responsibility of health professionals under the World Medical Association's Declaration of Helsinki.[61] The Declaration lists the information that should be provided in order to secure informed consent in paragraph 24. The World Health Organisation (WHO) has also published guidance on the process of seeking informed consent (including from children).[62]

Since each potential subject must be adequately informed of the aims and anticipated benefits and potential risks of the study, implementation of these requirements requires closer attention to be paid to the *purpose* of the experiments i.e. to their claimed aims and potential benefits and thus to *why* these experiments are being done. This requires further examination of the role of the proposed experiments in any future public health decision, so that members of the public can be fully informed about their purpose before giving their consent.

Role of the experiments in a public health decision

Ultimately, experimental releases of GM mosquitoes are intended to inform a decision about a potential public health intervention in the future. However, unlike clinical trials of medicines for example (which play a clear role in the approvals process by testing both efficacy and safety) there is no clearly established process for approval of a commercial-scale release of GM mosquitoes.

Thus, the types of information needed to make such a decision need to be considered at an early stage, so that experiments are not performed unnecessarily (for example, when it is already known that costs of commercial use would be prohibitive; or that some hazards cannot be characterised without a better knowledge of baseline conditions); and so that any experiments that are done genuinely contribute to reducing uncertainty about potential risks and benefits (as described above under 'step-by-step approach').

In the case of GM mosquitoes, the role of government differs significantly from the situation with GM crops where it is up to farmers to make a decision whether to plant a crop, taking into account additional factors (such as costs, liability for any harms and the availability of alternatives) that are not considered during the approvals process. To reach a fully informed decision on future releases, the Malaysian government will need to weigh up the pros and cons of releasing GM mosquitoes in a way which includes social, ethical and economic costs and also considers alternatives and other health priorities as well as public and expert opinions on these matters. It may also wish to see additional safeguards (such as legislation on liability) to be put in place.

It is worth noting that a large number of alternative dengue vector control strategies are currently under investigation: weighing up the pros and cons of these alternatives is likely to be relevant to any public health decision. Examples include the use of insecticide treated curtains and water container covers tested by WHO/TDR[63] and the potential use of a wide range of newly developed larvicides, including e.g. potash alum.[64]

Neither the aim of the company (to commercialise its product) nor the aim of the Cartegena Protocol (to protect biodiversity) are sufficient to ensure that the goal of protecting and improving public health is met. GeneWatch therefore recommends that the GMAC clarifies its views on the role of the experiments in its decision-making process and what questions it regards as needing to be answered (for example, regarding long-term risks, efficacy and cost-effectiveness) in order to provide the

necessary public information regarding the aims, purposes and justification for undertaking these experiments.

For further information contact:

Dr Helen Wallace
Director
GeneWatch UK
60 Lightwood Rd
Buxton
SK17 7BB
UK
Tel: +44-1298-24300
Email: helen.wallace@genewatch.org
Website: www.genewatch.org

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