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Open field release of a self-limiting transgenic *Aedes aegypti* mosquito strain to combat dengue – a structured risk-benefit analysis

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Abstract. Genetically modified mosquitoes carrying a repressible lethal trait have been developed as a possible means of controlling vector-borne diseases such as dengue fever, chikungunya, yellow fever and malaria. In Malaysia release of GM *Aedes aegypti* mosquitoes is being given serious consideration to control dengue, and a small scale open field release has recently taken place. The risks associated with this approach have been extensively discussed and documented, but there has been no similar attempt to dimension the associated benefits. This paper demonstrates the applicability of a semi-quantitative approach to risk-benefit analysis to compare risks and benefits of open field release GM mosquitoes taking into consideration environmental, health and socio-economic impacts.

Keywords: *Aedes*, benefit, dengue, field release, GM, mosquito, risk, transgenic.

INTRODUCTION

Dengue is a growing public health problem in the subtropics and tropics for which there is no vaccine or cure. There are 50-100 million new infections annually, and the incidence is increasing (Massad and Coutinho, 2011). The mosquito *Aedes aegypti* is the vector responsible for transmission of dengue, chikungunya and yellow fever. Genetically modified (GM) *Aedes aegypti* mosquitoes have been produced expressing a fluorescent marker gene and a repressible lethal trait known as RIDL ("Release of Insects carrying a Dominant Lethal") (Thomas *et al.*, 2000; Phuc *et al.*, 2007). RIDL mosquitoes contain a modification that causes their offspring to die, but the insects can live and reproduce normally when the larvae are fed a diet containing a supplement. The inserted genetic construct causes expression of a fluorescent marker gene (*DsRed*2), used to identify the transgenic mosquitoes, and a dominant tetracycline-repressible transcriptional activator that is toxic to the mosquitoes but can be repressed in the laboratory to allow the breeding population to be maintained. When RIDL males are released to mate with wild female mosquitoes, their progeny that inherit the RIDL gene do not survive to adulthood. Releases of RIDL males in large enough numbers over a sufficient time may suppress, or even eliminate, the target pest population.

Beech *et al.* (2009) published the results of a consultative workshop where the risks associated with the open field release of transgenic *Aedes aegypti* mosquitoes in Malaysia were considered. Patil *et al.* (2010) identified two additional hazards, though the overall risk associated with these was considered to be low. Nevertheless there remain significant gaps in our knowledge, particularly concerning transmission thresholds, since *Aedes aegypti* mosquitoes can continue to transmit dengue even at low population densities (Scott *et al.*, 2002). The RIDL approach needs to be considered in comparison with other intervention strategies, such as the use of insecticides or alternative transgenic approaches (Luz *et al.*, 2010). Medlock *et al.* (2009) compared the impact of various transgenic mosquito technologies on likely dengue virulence to humans and mosquitoes, and concluded that strategies that would increase the background mosquito mortality (such as RIDL) might increase virulence to mosquitoes, but would not impact on virulence to humans.

It has been reported that a small scale trial release of 6000 male transgenic *Aedes aegypti* mosquitoes of the strain OX513A took place in December 2010 at an uninhabited site in Malaysia (Tan, 2011; Nature Editorial, 2011). This followed a much larger field release in the Cayman Islands. However, if ongoing release of GM mosquitoes is to achieve broad acceptance as a means of combating dengue fever in Malaysia, it is important that methods are available to impartially analyse and communicate both the risks and benefits of such a strategy.

A methodology to undertake a semi-quantitative risk-benefit analysis for genetically modified organisms (GMOs) has been recently developed (Morris, 2011) and its applicability to the release of *Aedes aegypti* mosquitoes to combat

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dengue in Malaysia was briefly alluded to. In this paper the risk-benefit analysis is described in more detail.

Risk analysis vs risk-benefit analysis

In the medical field the concept of appropriate assessment of both risks and benefits associated with a treatment intervention is not new. Holden (2003) and Felli *et al*. (2009) have reviewed some of the available methodologies to undertake a risk-benefit analysis for a medicine. However in the ongoing debate about the safety of GMOs, the risk-benefit concept has not yet been adopted to any great extent.

The Cartagena Protocol on Biosafety (CPB) (Secretariat of the Convention on Biological Diversity 2000) is a legally binding international agreement that regulates transboundary movement of Living Modified Organisms (LMOs), more commonly referred to as GMOs. It is based on the Precautionary Principle, which focuses entirely on minimizing or eliminating risks, without consideration of corresponding benefits. In recent years there have been increasing calls for benefits to be considered as well as risks, if only to assist in determining what level of residual risk might be considered acceptable (Winter, 2008).

The precautionary approach has been adopted in the national biosafety legislation of the majority of countries that have acceded to the CPB, including Malaysia, as reflected in the Malaysian Biosafety Act 2007 (Ministry of Natural Resources and Environment, 2007). This Act makes reference only to risks and not benefits. Nevertheless, the objectives of this Act are stated to be "protecting human, plant and animal health, the environment and biological diversity", which might possibly not exclude considerations of benefits to any of these elements.

As long as individuals and societies focus only on risks, there is a danger that potentially beneficial technologies will not be introduced, the resulting inaction putting a brake on development. Moreover, in any decision to introduce (or not) a new technology, alternatives to action need to be considered and compared. Therefore a methodology for assessment of risks and benefits that is applicable to GMOs becomes extremely important to guide the decision making process.

The risk-benefit methodology

As described by Morris (2011), the methodology involves a number of analytical steps to arrive at a Risk-Benefit Score (RBS). Hazards (the potential of the organism to cause harm) and positive effects (the potential of an organism to cause beneficial effects or reduce adverse effects) should first be identified and prioritized in a scoping exercise, and grouped into categories such as Environment, Health, Agriculture, Food, Socio-economics etc. For each of these prioritized issues, scores are assigned for various criteria as outlined in Appendix 1.

The scores are then calculated within each category to achieve an overall score as demonstrated below for the Environment Score (ES). Each component (hazard or positive effect) is assigned a number within the category, which is reflected as a subscript in the equations below. The overall ES score is determined as the sum of the individual scores divided by the number of components evaluated and determined to have a non-zero score (n).

$$A1_1 * A2_1 = AT_1$$
$$B1_1 + B2_1 + B3_1 = BT_1$$
$$AT_1 * BT_1 * P_1 = ES_1$$
$$(ES_1 + ES_2 \ldots ES_n)/n = ES$$

This procedure is followed for each category of hazards and positive effects, to arrive at a Health Score (HS), Agriculture Score (AS), Food Score (FS) or Socio-economic Score (SS). A positive score indicates an overall benefit, whereas a negative score indicates an overall risk. Any other desired category may be included. Finally, the RBS is calculated as the sum of the individual category scores.

Thus the RBS provides an overall perspective on the balance between Risk (defined as the combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the consequences will occur) and Benefit (defined as the combination of the magnitude of the consequences of a positive effect, if it occurs, and the likelihood that the consequences will occur, i.e. the opposite of risk).

The risk-benefit methodology as applied to the open field release of transgenic **Aedes aegypti**

Beech *et al*. (2009) and Patil *et al*. (2010) identified a range of hazards and made an assessment of the overall risk associated with each hazard. This information was applied in the current paper, the hazards selected for inclusion in the risk-benefit assessment being largely based on those deemed by Beech *et al* to be associated with a non-negligible overall risk. Their assessment did not extend to a similar examination of positive effects or potential benefits, which have therefore been assessed here based purely on the views of the author.

An additional factor given consideration is the possibility that the GM mosquito intervention would not be sustained, and the remaining mosquitoes would then multiply and return to the population levels prior to the intervention. This could result in in exacerbation of future epidemics following loss of immunity in the human population. However such a scenario also applies to interventions with insecticides (Luz *et al*., 2010), and a combination of different interventions (of which RIDL could be one) is likely to be most successful. Since this is an issue that would arise from the withdrawal of the GMO rather than from its introduction, it is not included in Appendix II

It is a matter of some debate to what extent socio-economic considerations should be included in a risk analysis or risk-benefit analysis. Beech *et al* (2009) stated that socio-economic aspects were discussed but were not included in their paper, since they were outside a formal science-based risk assessment. Nevertheless, since the Malaysian Biosafety Act makes provision for the inclusion of socio-economic issues, it seems logical that they should be considered here.

The results of the risk-benefit analysis are shown in Appendix II, and demonstrate that the benefits outweigh the risks in all categories, with an overall positive RBS of 17.14. The scores differ somewhat from those in Morris (2011) because some additional factors were taken into consideration. Nevertheless, the overall trend remains the same.

It should be stressed that the risk-benefit analysis should ideally be undertaken by a panel of experts who would identify the most significant hazards and positive effects in a preliminary framing exercise, and would then proceed to assign scores to each of these. In the absence of such a process, the scoring cannot be regarded as definitive, and should not replace a full assessment under the Biosafety Act.

CONCLUSION

Few comprehensive studies have been undertaken to evaluate the impact of release of transgenic insects or other related technologies. The publications by Murphy *et al.* (2010) and others as summarized by Vasan (2010) primarily concern risks. The United States Department of Agriculture (USDA) published an Environmental Impact Statement on the use of genetically engineered fruit fly and pink bollworm in plant pest control programs (USDA, 2008). This very comprehensive document is complex and largely descriptive, emphasizing the potential value of a relatively simple decision support tool to facilitate comparison of risks and benefits. Moreover, it should be taken into consideration that many developing countries (where release of transgenic *Aedes aegypti* is most likely to occur) do not have the human or financial resources to produce such extensive documentation, but nevertheless must take decisions based on the evidence to hand.

The risk-benefit methodology provides a relatively simple means of assessing all relevant issues in a structured and transparent fashion. As such, it can be used as a decision support tool to assist regulators, but may also be used as part of a public engagement and risk-benefit communication exercise to assist communities to achieve a better understanding and reach consensus on the release of GM mosquitoes.

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APPENDIX I

Definition of criteria for assigning scores

Criterion	Scale	Description
A1. Importance of the impact	4	Important to the population or the environment as a whole, goes beyond national interests
	3	Important at national level to the population or the environment
	2	Important to areas or to population groups immediately outside the local condition
	1	Important to a small group of people or to a small location
	0	No importance or not relevant
A2. Magnitude of change	+3	Major positive effect
	+2	Significant improvement in status quo
	+1	Improvement in status quo
	0	No change to status quo
	-1	Negative change to status quo
	-2	Significant negative disadvantage or change
	-3	Major negative disadvantage or change
B1. Susceptibility of the environment or affected population	3	Extremely sensitive environment or population
	2	Some sensitivity in the environment or population
	1	No change/not applicable
B2. Reversibility of impact	3	Impact will change the environment or affected population irreversibly or restoration will last at least 10 years
	2	Reversible impact; the GMO can be easily withdrawn and the situation restored to the status quo
	1	No change/not applicable
B3. Cumulativeness/synergism of impact	3	The activity will have obvious cumulative or synergistic effects with other activities (e.g. other GMO events) in the same area
	2	May be some cumulative or synergistic effects but their effect is likely to be relatively small
	1	No cumulativeness/Not applicable
P. Probability or likelihood of occurrence[a]	1	Certain
	0.93	Almost certain
	0.75	Probable
	0.5	Chances about even
	0.3	Probably not
	0.07	Almost certainly not
	0	Impossible

APPENDIX II

Risk benefit assessment of open field release in Malaysia of male of *Aedes aegypti* mosquitoes (RIDL) containing a repressible lethal system that kills their progeny

Issues considered	A1	A2	B1	B2	B3	P	Score
ES							ES_{1-6}
Risks							
Negative effects on other organisms that feed on mosquitoes	3	-1	1	2	1	0.07	-0.84
Negative effects on plants that are pollinated by *Aedes aegypti*	3	0	1	2	1	0.07	0
A. albopictus population increases to take over the ecological niche of *A. aegypti*	3	0[a]	1	1	1	0.5	0
Increased *Aedes albopictus* population results in increased zoonotic disease transmission	1	-1	1	2	1	0.07	-0.28
Negative effect on water and soil quality from introduced proteins	3	-1	1	2	1	0.07	-0.84
Benefits							
Reduced need for insecticide spraying against mosquitoes	3	1	1	1	1	0.75	6.75
							ES=1.20
AS/FS							
Risks None foreseen *Benefits* None foreseen							
HS							HS_{1-2}
Risks							
Increased disease transmission if wild females become aggressive after mating with GM sterile males	1	-1	2	2	1	0.07	-0.35
Benefits							
Decreased incidence of dengue fever	3	2	2	2	1	0.75	22.5
							HS=+11.08

SS						SS_{1-3}
Risks						
Increased cost of dengue fever control due to need for sustained release of mosquitoes	3	-1	2	2	1	0.5	-7.5
Violation of terms of the Cartagena Protocol on advance informed consent[c]	1	-1	2	3	1	0.07	-0.42
Benefits							
Reduction in economic cost of dengue fever treatment	3	2	2	2	1	0.75	22.5
							SS=+4.86
					OVERALL RBS		**17.14**

[a] Both vectors carry similar diseases and occupy similar niches so change may be neither positive nor negative. However *A. albopictus* populations now present in South-east Asia are less susceptible to infection with dengue than are *A. aegypti* populations (Gratz, 2004)

[b] *A. albopictus* has been shown to transmit arboviruses to laboratory animals and birds (Gratz, 2004). However both *A. albopictus* and *A. aegypti* appear to prefer to feed on humans (Ponlawat and Harrington, 2005).

[c]This consideration is included because of the possibility that released GM mosquitoes could cross country borders.