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Letter to the Editor: Discussion on the proposed hypothetical risks in relation to open field release of a self-limiting transgenic *Aedes aegypti* mosquito strains to combat dengue

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Aedes aegypti is the major mosquito vector of dengue fever (DF), dengue haemoraghic fever (DHF), dengue shock syndrome (DSS) and chikungunya around the globe (Chaturvedi and Nagar, 2008). In spite of considerable efforts to control dengue and chikungunya, these diseases remain of considerable importance around the world and the absence of specific treatment or preventive vaccine for DF/CHIK has led to a search for new technologies for vector control. Successful and effective disease-vector control strategies have been very difficult to achieve using conventional methods, due to various factors including insecticide resistance, poor knowledge of the biology of the vectors, and also limited human capacity as all the breeding sites of mosquitoes are not easily accessible particularly *Ae. aegypti* which prefers to breed in clean water like flower pots, tree holes etc. To overcome these difficulties, it becomes necessary to apply selective, target-specific and site-specific control strategies based on increased knowledge of the biology of vector-pathogen-human interactions, on the improvement of existing control tools and on the development of new and innovative vector control tools and strategies. The use of innovative technologies like Genetically Modified Mosquitoes (GMM) within integrated control strategies is feasible, however for proper implementation studies on efficacy, safety for humans and the environment, ethical legal and social implications (ELSI) concerns and public acceptance should be properly addressed (Toure *et al.*, 2004; Vasan, 2009; Wilke *et al.*, 2009).

Earlier a UNDP–sponsored workshop on the risk assessment of transgenic insects (series – 1) was co-hosted in November 2008 by Malaysia's Ministry of Natural Resources and Environment, the Institute for Medical Research (IMR) under the ministry of Health Malaysia, and the Centre for Research in Biotechnology for Agriculture at the University of Malaya. The workshop extensively discussed the risks and benefits of three case studies: hypothetical field release of genetically modified fruit flies (Tephritidae sp.), pink bollworm (*Pectinophora gossypiella*) and mosquitoes (*Ae. aegypti*). The participants determined the potential hazards associated with these hypothetical trials, and then applied the tools of risk assessment and risk mitigation / management to determine the likelihood and consequences of the identified potential hazards, and to prepare an overall risk assessment

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(Beech *et al.*, 2009). The overall risk was ranked accordingly (Viz. High – Rank 1, Medium – Rank 2, Low – Rank 3, and Negligible – Rank 4). The risk assessment carried out is concerned to the use of a transgenic *Ae. aegypti* mosquito expressing a fluorescent marker gene (*DsRed*) and a repressible lethal trait (known as RIDL) in order to suppress the target field population of *Ae. aegypti* in Peninsular Malaysia (Thomas *et al.*, 2000; Phuc *et al.*, 2007 and Lee *et al.*, 2008). To create a pool of regional scientists well-trained in the assessment and management of biosafety issues and implementation of genetically modified disease vectors for the control of vector-borne diseases, courses on biosafety training related to potential release of genetically modified disease vectors are being conducted in Africa, Latin America and Asia and are sponsored by TDR. Recently a UNICEF / UNDP / World Bank / WHO – sponsored second Asian training course on "Biosafety for Human Health and the Environment in Relation to Potential Release of Genetically Modified Vectors" was hosted and organized on 22nd February – 5th March 2010 by the Centre for Research in Medical Entomology (CRME), Madurai, India, an Institute of Indian Council of Medical Research (ICMR). These 12 days of training were attended by 14 scientists working in the field of entomology, medical entomology, infectious diseases, law, social sciences, medicine, vector control and microbiology. The objectives of this Biosafety Training Course were:

1. To increase the awareness of Asian researchers and decision-makers of issues and challenges such as ethical, legal and social implications related to the development and implementation of this technology.

2. To ensure the feasibility and safety of genetically modified disease vectors in Asian countries.

3. To build capacity in Asia for the safe development and implementation of this technology.

During the training program an approach was made to add to the discussion and conclusions of the previous workshop on risk assessment mentioned by Beech *et al.* (2009) associated with the implementation of GM technology. In this brief note, we discussed the report of risk assessment in Beech *et al.* (2009) for few additional potential consequences, risk mitigation / management, overall risk and ranking in response against potential hazards, and in addition we proposed and discussed other possible potential hazards associated with the release of genetically modified mosquitoes (Appendix I).

Other than the risk assessment associated with the implementation of RIDL technology we also discussed potential hazards associated with the implementation of GM mosquitoes with a female-specific flightless phenotype for population control (Fu *et al.*, 2010) within an integrated program of mosquito management (Appendix II).

The trainees were divided into two groups of 7 in a way that ensured balance in terms of gender. Each group independently prepared and discussed risk assessments for the potential hazards identified by Beech *et al.* (2009) and additional potential hazards proposed by the groups. The outputs from each group were then discussed, combined and harmonized into a single document and summarized (Appendix I and II).

Additional hazards identified to supplement the UNDP workshop output

1. ***Multiple mating behaviour of female Ae. aegypti with sterile males or wild males or both:*** The female *Ae. aegypti* mosquito mates once in her lifetime and stores the sperm in the spermathecae. Release of sterile males in the environment could lead to competition between sterile and wild males. As sterile males are released at a much greater ratio than that of wild males (10:1 or greater) there is much less chance for wild male to come across a female partner for mating leading to sex-starved wild males. However it is intrinsic property of the male insects to search for female partner for mating which may lead and/or increase the chances of forced mating by wild males with the wild females which have been already mated with the sterile males. This behavioural change may affect the efficacy of the SIT program and may lead to multiple mating behavioural activity in female mosquitoes. We suggest that forced mating experiments be examined under laboratory conditions. This can be done by allowing one wild female to mate with a sterile male and then introduce it into cage containing a known number of wild males and look for any possible forced mating. If forced mating occurs then one should look for the successful intrusion of sperm into the female genital tract / spermatheca. Further successful intrusion of sperm, if any, by the wild male mosquito into the spermatheca of the mated (by sterile male) female will lead to mixing of sperms in the spermatheca which will lead to competition between the fertile and 'infertile' sperms. Studies into this possibility are suggested.

2. ***Presence of tetracycline in the environment:*** This was thought by participants in the training to be of low risk. However, many commercially available animal feeds contain tetracycline which may lead to contamination of *Ae. aegypti* breeding sites leading to successful emergence of adult mosquitoes and affect the efficacy of the RIDL technology.

CONCLUSION

The ethical principle dictates that we should be reasonably cautious about premature use of a technology before potential risks are understood (Macer, 2007). Some have advocated a total precautionary principle for genetic engineering, which means that no technology with any known risk should be attempted (Ho, 1998). The Cartagena Protocol on Biosafety, an international, legally binding agreement that regulates international movement of living modified organisms (LMOs), advises this extreme caution (CBD, 2000). Since no human action can be guaranteed to have zero risk, in practice, these principles are used to assess the relative safety of technology and are central to any public health program (Callahan, 2002). In view of this the training participants have discussed the potential hazards and risks associated with the implementation of the GM technol ogy with reference to RIDL technology and female-specific flightless phenotype for mosquito control. We reviewed the list of risks in Beech *et al.* (2009) and our group identified two additional risks for RIDL technology to add to the overall risk register. Both of these were related to the potential of the overall efficacy of the product to be compromised, although were thought overall to have low risk potential. In addition an attempt was made to find out the potential hazards associated with the implementation of GM mosquitoes with a female-specific flightless phenotype for population control within an integrated program of mosquito management. The groups identified eight potential hazards and discussed which were thought overall to have negligible risk.

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Appendix I. Updated version of Appendix 1 in Beech *et al.* (2009) for a bisex lethal strain.

Sl. No.	Potential hazard	Potential consequence	Risk mitigation / management	Overall risk	Rank
1 (3)	Cross mating with other mosquito species	Potential gene transfer to other mosquito species.	In nature cross species mating is rare. Experiments on the cross mating under laboratory conditions between *Ae. aegypti* and *Ae. albopictus* and vise-versa have shown that the progeny does not survive.	Negligible	4
2 (4)	(GM) Mosquito lives longer	Increased mating opportunities.	In case GE mosquitoes live longer, it would be beneficial as chances of wild female mosquitoes mating with GE males would be higher. Further frequency of sterile males releases will be reduced if the GE mosquito lives longer increaseing the efficacy of the SIT technology and also making it cost effective.	Negligible	4
3 (5)	Wild females become aggressive after mating with GE sterile males	Increased biting activities and increased disease transmission.	Mating is unlikely to induce changes biting and feeding behaviour.	Negligible	4
4 (9)	Increased resistance to insecticide / fogging	More GM sterile male mosquitoes survive in the environment. Potential for increased disease transmission by introgress of resistance determinants into local female population.	There are no experimental evidence. Further the sterile males released are reared under laboratory conditions and they are expected to be more susceptible to insecticides rather than develop resistance. However if the sterile males are more resistant to insecticides then it will be beneficial as this will help to implement conventional methods (spraying insecticides) and GM technology to go hand in hand.	Negligible	4
5 (12)	Stability of gene construct	Failure to reduce pest population.	Even though laboratory evidences have shown stability of the gene construct for several generations, they have to be cross checked frequently / regularly in order to avoid any negative consequences.	Low	3
6 (17)	Increase in host range	More animals are bitten by mosquito and may harbor other pathogens from animals.	As the release of sterile males would increase chances of all wild females being mated, there will be an urge to feed after mating which might lead to increase in host range in case the number of preferred hosts are not adequate. However field studies in this aspect is necessary in the future.	Negligible	4
7 (19)	The reduction in survival of target organisms	Reduction in *Ae. aegypti*. *Ae. albopictus* will take over. Increased possibility of incidence of CHIK.	The reduction / eradication of target organism / vector is very unlikely using only GM technology. Only an integrated approach for vector control including the SIT can lead to suppression of target population below potential disease transmission threshold level. However control measures for both species is most essential to control chikungunya.	Low	3

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Sl. No.	Potential hazard	Potential consequence	Risk mitigation / management	Overall risk	Rank
8 (23)	Effect on the existing ecosystem	Food chain affected	There are more than 3000 sp of mosquitoes globally and quite a number of species cohabiting in any said area. Therefore, any other insect or mosquito species per se may fill the gap in the food chain/ecosystem. Further *Ae. aegypti* is not a native species.	Low	**3**
9 (24)	Accidental release of mosquito	No recall procedure and mosquitoes enter the environment.	RIDL technology is a self limiting technology and any accidental release has negligible risk. However staff should be trained and prepared for management of accidental release / escape of GM mosquitoes.	Low	**3**
10 (29)	Working with transgenic seen as a work hazard	Difficult to recruit manpower for experiments.	Although the risk involved in working with RIDL technology is negligible. Good Laboratory Practice (GLP) and Good Management Practice (GMP) must be followed.	Low	**3**
11 (30)	Increase in *Aedes albopictus* population	Increase in possiblity of zoonotic transimission.	There is no evidence to suggest that increase / decrease of *Ae. albopictus* population is related to increase / decrease of *Ae. aegypti* population as there is interspecies competition between these two species. However needs further evaluation in future.	Medium	**2**
12 (31)	Change in water and soil quality		Further studies required but highly improbable	Negligible	**4**
Additional hazards identified to supplement the UNDP workshop output, and/or relevant to a hypothetical release in Madurai…					
13	Multiple mating of wild female with sterile / wild male	May affect the efficacy of SIT programme.	The female mosquitoes usually mate once. However if the mated (by sterile males) females come across the wild males, there is a chance of female going for second mating by wild males or forced mating may take place as the wild males will be in search of female partner. This may induce behavioural changes leading to mutiple mating behaviour. Further studies on the progeny of multiple mated female under laboratory conditions are necessary with respect to sperm competition. If this behavioural change occurs then it will affect the efficacy of SIT programme but no risk to human	Low	3
14	Presence of tetracycline in environment	Effect of SIT programme.	Certain fish food, chick food and other animal food contain tetracycline. Presence of the tetracycline in *Ae. aegypti* breeding sites may reduce the effcicacy of RIDL technology.	Low	3

The figures in the parentheses represent the serial number of potential hazard in Beech *et al.* 2009.

Appendix II. Updated version of Appendix 1 in Beech *et al.* (2009) for a female-specific flightless strain.

Sl. No.	Potential hazard	Potential consequence	Risk mitigation / management	Overall risk	Rank
1	Flightless females can mate with wild males.	Produce healthy offsprings that will transmit diseases.	Flightless females are unable to attract and mate with males as courtship and mating depend on the wing oscillation "song" and further mating usually takes place during flight.	Negligible	4
2	Flightless females can bite people.	Transmit dengue / chikungunya disease.	Flightless female mosquito may either die in water after their emergence or may get eaten by other predatory insects. Further the period required to get infected with the dengue virus and chikungunya virus is around 7 days after feeding with the infected person and is rarely possible to transmit disease as flightless mosquito cannot survive for 7 days in the environment.	Negligible	4
3	Flightless females will die in the water leading to contamination.	Contamination of stored water causing health hazards.	No evidence and very unlikely to pose health hazards.	Negligible	4
4	Flightless females are eaten by other insects.	Horizontal transfer of genes.	No evidence.	Negligible	4
5	Change in mating behaviour of males.	May affect the SIT program.	No evidence. Mosquitoes mate during flight.	Negligible	4
6	Increased flight range of male mosquitoes to find active females.	Increased dispersion of mosquito population.	Do not pose any health hazard.	Negligible	4
7	Flightless females may be susceptible to other diseases such as AIDS.	May transmit AIDS	Not likely	Negligible	4
8	The released males may not be competent enough to mate with wild males where the GMM are intended to be released.	Failure of technology in reducing the target population.	Experiments on mating competiveness with the strain of locality, where the GMM are intended to be released might be helpful.	Low	3