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Risk analysis of a hypothetical open field release of a self-limiting transgenic *Aedes aegypti* mosquito strain to combat dengue

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Abstract. 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. This 3-day workshop was attended by 70 scientists working in the fields of biosafety, entomology and medical entomology, infectious diseases, law, medicine, natural resources and the environment, vector control and virology. This workshop is one of the initiatives under the project Capacity Building for Implementation of Malaysia's Biosafety Act 2007, which has the objective to help consolidate Malaysia's national capacity for the implementation of the Cartagena Protocol on Biosafety as well as the National Biosafety Act of 2007. 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 (*Aedes aegypti*). This paper discusses the methodology of the workshop and the output of the mosquito case study, where participants were asked to determine potential hazards associated with these hypothetical trials, and then apply the tools of risk assessment and risk management to determine the likelihood and consequence of the identified potential hazards, and thus prepare an overall risk assessment.

Keywords: *Aedes*; Dengue; Field Release; Risk Assessment; Risk Management; Transgenic Insects

BACKGROUND

Genetic transformation of disease-spreading mosquitoes has the potential to provide new opportunities for effective vector control against diseases such as dengue, chikungunya, and malaria. For example, the mosquito *Aedes aegypti* (vector of dengue, chikungunya and yellow fever) was transformed in 1998 (Coates, 1998; Jasinskiene *et al.*, 1998) and the malaria vector *Anopheles stephensi* was transformed in 2000 (Catteruccia *et al.*, 2000). A strain (OX513A) of *Aedes aegypti* exhibiting repressible lethality (Phuc *et al.*, 2007) has shown promising results in laboratory and semi-field evaluation (Lee *et al.*, 2008; Khongtak *et al.*, 2009). However, transgenic mosquitoes must be assessed for their potential impact on the environment and human health before their potential opportunities can be realised. How this can be done has been widely debated for over a decade, for instance, in 1991 by the Vector Biology Network sponsored by the World Health Organisation and the McArthur Foundation (WHO/TDR, 1991), in the EU FRONTIS Workshops in 2002 and 2004 (Takken *et al.*, 2002; Knols *et al.*, 2004) at an International Atomic Energy Authority (IAEA) workshop (IAEA, 2006), and recently in the WHO/FNIH Technical Consultation on Genetically Modified Mosquitoes (WHO, 2009).

A risk analysis framework, encompassing risk assessment, risk management and risk communication must be developed and put in place to adequately assess the impact of any release of transgenic mosquitoes for vector control and ultimately disease control. For disease endemic countries (DECs) to take full advantage of the potential of these new tools for vector management it is important that each DEC starts to develop the necessary regulations and the expertise to assess the potential risks associated with release of transgenic insects and appropriate risk management methods that might be required on a case-by-case basis (Vasan, 2009; Beech *et al.*, 2009).

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Malaysia is one such DEC that is taking pioneering steps to build the necessary expertise to evaluate and potentially deploy promising tools resulting from transgenic insect strategies, with the goal of reducing their disease burden, particularly due to the twin menace of dengue fever and chikungunya that have resurged to high levels this decade (Lee *et al*., 2008). The project Capacity Building for Implementation of Malaysia's Biosafety Act 2007 is led by the Conservation and Environmental Management Division under the Malaysian Ministry of Natural Resource and Environment with support from the United Nations Development Program. The objective of this project is to consolidate Malaysia's national capacity for the implementation of the Cartagena Protocol on Biosafety as well as the Malaysian National Biosafety Act 2007. One of the main components of the project is to specifically develop national capacities in biosafety required to carry out risk assessments with appropriate scientific and technical skills.

The Capacity Building project in conjunction with the Institute for Medical Research, Kuala Lumpur and the Centre for Research in Biotechnology for Agriculture at the University of Malaya organised a Workshop on the Risk Assessment of Transgenic Insects (Series 1) in order to meet the goals in building capacity amongst regulators and scientists to undertake science-based risk assessment (which is the first and most crucial part of risk analysis) in this new and rapidly growing field. It is understood that this was the first science-based risk assessment workshop on transgenic mosquitoes to take place anywhere in the world (NRE, 2009), and perhaps the second instance that such a systematic exercise was carried out on transgenic insects anywhere in the world. The first instance was when the United States Department of Agriculture pioneered risk assessments in the field of transgenic insects and published two Environmental Assessments (USDA, 2001; USDA, 2006) and an Environmental Impact Statement (USDA, 2008; APHIS, 2009) on genetically engineered Fruit Fly and Pink Bollworm. The EIS recommended that genetically engineered Fruit Fly and Pink Bollworm be integrated into APHIS plant pest control programs as it is an environmentally preferable alternative (Rose, 2009).

Workshop Objectives

The objectives of the Workshop on the Risk Assessment of Transgenic Insects (Series-1) were to:

a) Enhance knowledge of participants on the Convention of Biological Diversity (CBD) and the Cartagena Protocol on Biosafety (CPB), the Malaysian Biosafety Act 2007 and accompanying implementing regulations that are being drafted, as well as their role and obligations as research and development organisations, regulators and institutional biosafety committee members;

b) Build the capacity of participants in undertaking risk analysis of transgenic insects, and build capacity in evaluating risk analysis in order to facilitate decision making under the CPB;

c) Review current models for risk analysis applied to transgenic insects;

d) Explore experiences and lessons learned from other risk assessments, and implementing risk management and communication measures, along with social, ethical and cultural issues.

Workshop Methodology

This intensive workshop took place over three days (13-15 November 2008) at the Hilton Petaling Jaya Hotel, and was attended by 70 scientists and decision makers in Malaysia, along with experienced resource trainers from India, Malaysia, UK and USA. The academic fields represented at the meeting included biosafety, entomology and medical entomology, infectious diseases, law, medicine, natural resources and the environment, vector control and virology.

Risk analysis is used in a range of disciplines, including but not exclusively, the food industry (FAO/WHO,1997), environmental decision making (Jardine *et al*., 2003), chemical assessment (EPA, 2000; Arendt and Lorenzo, 2000), and genetically modified crops (Hill and Sendashonga, 2003; Raybould, 2006; Nickson, 2008). It is a term used to describe a wide range of methods, both formal and informal and often has multiple interpretations depending on the audience. However, the most usual interpretation is a formal way to characterise potential adverse impacts associated with a particular event or activity. It is usually science-based, case-by-case, taking into account available data and other relevant information such as host biology, information on the receiving environment, and the scope and scale of the activity. Each transgenic insect strain will have a different risk profile and therefore requires case-by-case risk analysis. Science-based risk assessment comprises of a number of steps, as summarised in Table 1.

Table 1: Steps in Science-based Risk Assessment

1. Problem formulation
2. Hazard identification
3. Evaluation of the likelihood of the hazard being realised
4. Evaluation of the consequences if the hazard is realised
5. Estimation of the "raw" risk (hazard x likelihood x consequence)
6. Application of risk management strategies to control "raw risks" identified
7. Determination of the overall risk

Risk assessment can be qualitative, semi-quantitative or quantitative, the choice often depending on the amount and quality of available data, the complexity of the risk under consideration, and the level of uncertainty concerning the potential risk. In some cases, where the scientific knowledge base is limited there will be scientific uncertainty. The degree of scientific uncertainty might lead towards a qualitative risk assessment, rather than a quantitative one. In all cases the aim is to have a repeatable, systematic and structured approach to risk evaluation.

In order to bring the workshop to a common interpretation of risk analysis and the status of transgenic insects, presentations were made by resource trainers on a variety of topics: the Cartagena Protocol on Biosafety and the Malaysian National Biosafety Act 2007, and the role of research and development organisations in their implementation; transgenic insects and their importance to public health and agriculture; Risk assessment, Risk management and Risk communication; social, ethical and cultural considerations; step-wise approaches to the programmatic use of transgenic insects; safety issues involving transgenic insects; molecular biology and characterisation of transgenic insects, etc. Risk assessment and risk management were described, with risk management being introduced as defining options for risk treatment, balancing the degree of protective measures required with the costs and effectiveness of applying them, as well as discussing the concept that risks could be accepted, transferred, mitigated or avoided, according to well established paradigms (Turner, 1999).

It was stressed that risk assessment is not a static process but an iterative process as details may need to be revisited due to findings in other components of the assessment, and also if additional new findings come to light during further experimentation or programmatic use. This principle is well-established in risk assessment disciplines. Perhaps the least time at this particular workshop was spent on the topic of risk communication, although the workshop itself and the publication of the output could be seen as such an exercise, and a follow-up workshop could address this aspect in detail. The Scientific Steering Committee of the European Commission (EC, 2000) has suggested that expressing the results of risk assessment in a user-friendly format such as risk ranking, comparison with alternatives, and using risk benefit analysis is a useful way of communicating risk.

Participants were trained on the concept of a risk assessment matrix to formalise both the process and the output. As mentioned in the introduction, the United States Department of Agriculture (USDA) has pioneered risk analysis of genetically engineered insects and produced two Environmental Assessments for field trials of genetically engineered pink bollworm (*Pectinophora gossypiella*) and an Environmental Impact Statement (EIS) on the use of genetically engineered Fruit Fly (*Tephritid* sp.) and Pink Bollworm in APHIS Plant Pest Control Programs (USDA, 2008). These existing risk assessments were presented and discussed, along with their use as training materials for the case studies on fruit fly and pink bollworm.

The overall group was divided into four working groups who independently prepared a risk assessment for the proposed case studies. These working groups were created in a way that ensured reasonable balance in terms of gender, expertise, experience, etc., and care was taken to keep line mangers/bosses in different working groups from their subordinates (details in the Appendix). The working groups were asked to play the role of a concerned Non Governmental Organisation (NGO) to determine any potential or theoretical hazards associated with a hypothetical field release of transgenic *Aedes aegypti* in a local tropical environment where dengue is endemic. In a further session they applied the tools of risk assessment and risk management as an Independent Biosafety Review Board in order to determine the likelihood and consequence of the identified potential hazards. The workshop did not have sufficient time to allow the separation of likelihood and consequence, or the uncertainty of the risk occurring for each potential hazard identified, so participants listed the consequence of the hazard as if it had occurred. Once potential consequences had been identified they applied risk management measures, as appropriate, to control the potential risk. After completing this process, they were asked to assign an overall ranking to the potential risk.

For each risk, the permitted values of rank were 1 (very important) or 2 (important) or 3 (somewhat important) or 4 (not important). The output from each of the four separate groups was then combined and harmonised into a single document by the resource trainers and organisers (co-authors of this paper), from which the key risks could be identified. For the sake of transparency and pedagogy, the raw output of each working group is available as an Appendix in the online version of the article on the journal's website.

Risk assessment case studies

Three risk assessment case studies were assessed, building the knowledge base of the participants. The first case study was based on transgenic Tephritid fruit flies that contained a marker gene from the Green Fluorescent Protein (GFP) super family and were irradiated using conventional and well-characterised sterile insect technique (SIT) techniques. The second case study was that of pink bollworm (*Pectinophora gossypiella*), a major insect pest of cotton, also containing a fluorescent marker gene (DsRed) and similarly irradiated using SIT methods. The third case study, the risk assessment of which is reported here, was that of transgenic *Aedes aegypti* mosquitoes expressing a fluorescent marker gene (DsRed) and a repressible lethal system known as RIDL (Thomas *et al.*, 2000; Phuc *et al.*, 2007; Lee *et al.*, 2008; Khongtak *et al.*, 2009) for a hypothetical large scale open field release in Peninsular Malaysia.

Aedes aegypti case study

The risk assessment presented here is for the use of a transgenic *Aedes aegypti* mosquito expressing a fluorescent marker gene and a repressible lethal trait in order to suppress the

target field population of *Aedes aegypti* in Peninsular Malaysia. This risk assessment is specific to this mosquito/trait combination in the particular receiving environment and cannot be extrapolated for other releases in different environments; however the general framework of the risk analysis and the process is valid for other assessments and participants were encouraged to use it after the workshop with appropriate modifications. The risk assessment produced by the four groups at the workshop was harmonised into one document by the resource trainers and organisers and is given in the table below:

Conclusions of the *Aedes aegypti* case study
For a hypothetical large scale field release of a transgenic *Aedes aegypti* mosquito expressing a marker gene and a repressible lethal gene, in a specific environment when evaluated on this specific basis, the overall conclusion by participants of the workshop was that the release would be of negligible risk to human health or the environment, with a rank of 4 (not important). However, within the risk analysis the following potential risks were identified as low risk or somewhat important (3):
1. Ecological niche replacement and potential increase of disease transmission by *Aedes albopictus.* (Lines 19, 30). This is likely to happen only over an extended time period, rather than in the context of a single experiment and is a potential risk that can be monitored over time. It was suggested that this could also be addressed by having a genetically modified *Aedes albopictus* available to reduce local *Aedes albopictus* populations.

2. Alteration of food chains or webs by the eradication of *Aedes aegypti.* (Lines 23, 28) This was thought to be low risk due to the fact that *Aedes aegypti* is not native to Asia, and there are other mosquito species on which predators and prey can feed, and that eradication of *Aedes aegypti* using this technique is probably impossible. It will be possible however, to reduce the numbers of *Aedes aegypti* to below a disease transmission threshold.

3. The potential for the transgenic *Aedes aegypti* to be less susceptible to insecticides used in control regimes and transfer such reduced susceptibility to the wild population (Line 9). It was suggested that information needs to be gathered to determine if the transgenic *Aedes aegypti* had similar susceptibilities to insecticides currently used in control regimes as the wild type. Such information will inform the risk assessment as to the ability to control the transgenic *Aedes aegypti* with conventional insecticides if required.

4. The potential for soil and water quality to be affected. (Line 31).This is not an issue that would prevent release, but it was recommended that some further information should be obtained. Soil quality is very unlikely to be affected by the release of these transgenic mosquitoes as the proteins will be rapidly broken down in GI tracts of mammals and predators and prey. Additionally, the genes incorporated into the mosquitoes are already present in the environment. Water quality needs further definition and an endpoint to be identified before studies could be performed, i.e. which water will be examined (rivers, streams or drinking water in vessels that might be mosquito breeding sites?) and what quality parameters will be tested? Impacts on soil and water from this release are likely to be much less than those from the use of pesticides to control the mosquito, which are often broad spectrum and affect non-target organisms as well, and consequently the utility of obtaining further information in regard to its impact on the risk analysis should be considered.

Other socio-economic aspects of the hypothetical release were also discussed by the groups but these are not included since they were outside a formal science-based risk assessment. If such a trial were to take place these issues could be dealt with by an ethics committee, or an overarching committee that could consider the socio-economic impact alongside the scientific risk assessment in a risk/benefit scenario.

DISCUSSION

Our experience was that participants in similar future workshops need hands on training to distinguish between hazard and risk, hazard and consequence, uncertainty analysis, risk assessment vs. risk management, and science based risk analysis (which includes risk assessment (RA), risk management (RM) and risk communication (RC)) versus other issues/concerns pertaining to ethical, social, cultural (ESC) aspects. Whilst ESC issues are valid concerns and should be addressed, science based risk assessment is not an appropriate place to do this (Vasan, 2009), although some authors have suggested including these issues in the risk assessment for greater transparency and to help allay acceptance issues. (Lavery, 2009; Angulo and Gilna, 2008; de Melo-Martin and Meghani, 2008).

In a group with a diverse background, as in the Malaysian workshop, only by the end of the first day could we bring everyone to same understanding, regarding the definitions and the differences between the types of activity (RA, RM, and RC). It required another day and several practice sessions with actual case studies to clarify risk analysis to the point that a new risk assessment with a different organism *(Aedes aegypti*) could be attempted. Additionally as there is potentially no end to the list of all the information one can evaluate the risk analysis must define what is necessary to know for risk assessment against what would be nice to know, and that the analysis must have a specific measurable endpoint. It is important to note that it was useful to have experts in the groups who understood the biology of the organisms under review, the genetic modification that has been introduced into the organisms, and laboratory data generated on the organisms, so that questions could be addressed during the group's discussions. One of the rec-

ommendations of the groups, in the post-course feedback, was to have a resource trainer within the group who could serve to answer such questions as and when they arose. The course evaluation report is included on the website of the journal for transparency and to assist others in preparing such courses. Generally by the end of second day, most participants were able to distinguish between these issues and felt comfortable with using risk analysis themselves and went on to produce the risk assessment in this document.

Future workshops could focus on Ethical, Social and Cultural issues related to release of genetically modified insects as well as further in depth scientific risk analysis, where there should be more time to focus on the likelihood and magnitude of potential hazards being realised, along with the estimation of uncertainty around the risks. In future workshops, more time could also be spent on risk management, weighing risks and benefits, and risk communication.

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APPENDIX 1

	Potential Hazard	Potential Consequence	Risk mitigation/management	Overall risk	Ranking
1	Male starts to bite	Increased transmission of disease	Males do not have morphology to bite. The anatomical structure of the mouthparts and the anatomical structure of the stomach prevent are incompatible with males having the capacity to bite. Additionally anti–coagulant secretions are required for biting and this has not been observed in males	Negligible	4
2	Biting period (frequency and peak biting period) extended	Increased transmission of disease	Males cannot bite. Bionomic equivalence of females demonstrated that female GM mosquitoes are the same as the wild type female	Negligible	4
3	Cross mating with other mosquito species	Potential gene transfer to other mosquito species	Biological data from experiments conducted and literature shows that cross-species mating results in non-viable progeny. Existing data shows there is reproductive isolation between species due to the structure of the genitalia (e.g. between *Aedes* and *Culex*)	Negligible	
4	(GM) Mosquito lives longer	Increased mating opportunities	Although theoretically possible, the bionomic studies of the RIDL mosquito show that it is not significantly different from wild type *A. aegypti*, with only a slight decrease in the longevity of the lifespan (2/3days) in the laboratory	Negligible	4
5	Wild females become aggressive after mating with GM sterile males	Increased biting activities and increased disease transmission	Currently no evidence from laboratory studies. Lab observations indicate absence of such behaviour. If more dengue cases are reported in an area, then emergency response plans can be implemented.	Low	3
6	Able to escape predators(both larvae and adults)	Increased numbers of mosquitoes in environment Increased disease transmission	The RIDL mosquitoes have shown normal behaviour and morphology in comparison to the wild type. Additionally the repressible lethality trait would have to fail to prevent the reduction of mosquito numbers in the environment	Negligible	4

7	GM sterile male mates more than once	Potential to increase numbers of mosquitoes in the environment	Normal biological processes for male mating have been observed for both GM and wild type males. It is usual behaviour for the male to mate more than once. The number of times the male mates has no impact on the number of mosquitoes in the environment. The reproductive potential of the population is limited by female numbers/fecundity etc	Negligible	4
8	Released male can self replicate	Increased numbers of mosquitoes in environment Male becomes established in environment	No scientific evidence that this happens in insects	Negligible	4
9	Increased resistance to insecticide/fogging	More GM sterile male mosquitoes survive in the environment. Potential for increased disease transmission by intorgress of resistance determinants into local female population	Scientific evidence shows no such difference as both RIDL GM and wild type are still susceptible to insecticide as expected because the genetic modification involves the mechanism for lethality, and not insecticide resistance. GM sterile male mosquitoes likely to be less fit that wild type in the environment and therefore more vunerable to insecticides. Insecticide resistance of GM sterile males alone would not lead to increased numbers of vectors or of disease transmission. Studies recommended examining the susceptibility to insecticides	Low	3
10	Vectorial capacity enhanced by genetic modification	Increase of other diseases enhanced by genetic modification	Males don't carry the virus. Current laboratory evidence indicates that the female GM mosquito vectorial capacity is still the same as the wild type, however the ability to transmit other pathogens is unknown. Scientific evidence shows that diseases are vector species –specific. Increase in other diseases would require the breakdown of the genetic construct (loss of lethality) without the loss of hypothetical modified vectorial capacity trait	Negligible	4

11	Infection with multiple of viruses	Causing more danger to human	Males don't carry viruses and there are no genes in the construct to alter function or morphology of the insects. Scientific evidence shows that it is not possible for wild–type *A. aegypti* to carry both dengue and CHK as when lab-fed with both, only one virus is found. Additional studies recommended to determine if this is the case for the GM mosquitoes	Negligible	4
12	Stability of gene construct	Failure to reduce pest population	Studies show that the gene is stable for over 50 generations. Transposon gene is immobilised and specific. No viable progeny are produced. An increase in the population is not likely from the release of GM *A. aegytpi* in the environment, merely a failure to reduce it	Negligible	4
13	Increase the population of mosquitoes	Higher incidence of dengue & chikungunya/yellow fever	Male mosquitoes don't bite or carry virus. Release of GM mosquitoes suppresses subsequent generations of the existing population	Negligible	4
14	Increase the size of mosquito	Painful bites, penetrate mosquito net	Males don't bite or carry virus. Progeny will not survive. Bionomic studies show that GM sterile males/females not different from wild type individuals. Lab studies show there is no physical difference in size between the RIDL mosquito and the wild type	Negligible	4
15	Increase number of eggs laid by wild type females	Increase the population	Mortality in 97% of progeny from females which mate with GE males. RIDL gene causes premature death of larvae	Negligible	4
16	Become heat resistant	Egg can survive for many years Adult mosquitoes survive at higher temperatures and spread to new areas.	There is no difference in the inherent characteristics of both the RIDL and the wild type mosquito in lab studies. Bionomic studies show that there is 97% mortality in progeny that hatched	Negligible	4
17	Increase in host range	More animals are bitten by mosquito	Male mosquitoes do not bite animals or humans as they do not possess the mouthparts to do so or the stomach morphology	Negligible	4
18	Larvae can survive without water	Increase mosquito population	*Aedes* larvae are aquatic — need water to survive and feed. Therefore larvae will die prematurely without water. There is no morphological or physical difference between the RIDL strain and the wild-type strain from laboratory studies	Negligible	4

19	The reduction in survival of target organism	Reduction in *A. aegypti, A. albopictus* will take over. Increased possibility of incidence of CHK	Eradication of *A. aegypti* is highly unlikely – only population suppression is likely. Long term strategy to control both species is desirable	Low	3
20	Take up more blood in bite	Possibility of anaphylactic shock	Males don't bite and take in blood, only females bite	Very negligible	4
21	Allergy to tetracycline in human	Anaphylactic shock and side effect of tetracycline	Tetracycline is in the larval feeds at the laboratory only. Adult mosquitoes do not have tetracycline. It is a photosensitive substance that breaks down readily and will not accumulate in the environment. Workers in the laboratory follow SOP's and wear protective equipment	Negligible	4
22	Horizontal gene transfer to humans	Cause death or shorten life of human	No scientific evidence. Mosquitoes do not transfer their genes due to species specific mating barriers. Strain has been demonstrated as stable for over 50 generations. Transposons are disabled and not sensitive to exogenous transposase. Remote chance that transgene is transferred to microbes in mosquitoes	Negligible	4
23	Effect of the existing ecosystem	Food chain affected	*A. aegypti* not native to Malaysia, and therefore there are alternative food sources and other mosquitoes species on which predators/ prey can feed	Low	3
24	Accidental release mosquito	No recall procedure and mosquitoes enter the environment	RIDL mosquitoes that mate with wild females will produce progeny that die in the absence of tetracycline. SOP and lab practices and staff training developed to limit accidental release although the potential likelihood of accidental release happening could be high. An emergency response plan is developed to deal with these issues	Negligible	4
25	Become an endangered species in 10-20 years time	Disruption of balance in ecosystem	Maintain lab strains for R&D. Very difficult to totally wipe out *A. aegypti* in Penninsular Malaysia. Other mosquitoes will remain in the environment as food sources for predators and prey	Negligible	4
26	Increase of allergies	Increase of asthmatic cases	No scientific evidence	Negligible	4
27	Male mosquito behaves as a pollinator and transfers gene(s) to plants	Humans can get ingest the genes within the mosquito by eating plants.	Male mosquitoes will seek out nectar sources and do pollinate some select plants, such as *Habaneria obtusata* (bog orchid). This plant is not present in Malaysia. No scientific evidence that insect pollinators transfer genes to plants.. Behaviourally the RIDL mosquito behaves as the wild type mosquito in laboratory studies. Humans digest DNA/protein in their GI tract	Negligible	4

28	Dead larvae	Affect aquatic industry	Fish and other aquatic organisms already eat mosquito larvae, this would increase the amount of food for the fish and potentially increase fish health. Consider a fish or other predator study to look for adverse/or health effects	Negligible	4
29	Working with transgenic seen as a work hazard	Difficult to recruit manpower for experiments	Biosafety procedures, intrinsic bio-containment, SOPs in place and trained staff	Negligible	4
30	Increase in *A. albopictus* population	Increase in possibility of zoonotic transmission	No evidence of dengue fever outside of humans or primates. Consider developing a GM *A. albopictus* to reduce the population and therefore disease transmission	Medium	2
31	Change in water and soil quality	Environment affected	Introduced proteins are digested with GI tracts of mammals and insects. Consider studies on soil and water quality post release of mosquitoes	Low	3

****APPENDIX 2**

1	Sazaly Abu Bakar	University of Malaya
2	Mohd Sofian Azirun	University of Malaya
3	Johari Haji Surin	University of Malaya
4	Norma Yusoff	University of Malaya
5	Fauziah Abdullah	University of Malaya
6	Fong Mun Yik	University of Malaya
7	Zulqarnain Mohamed	University of Malaya
8	Yusmin Yusof	University of Malaya
9	Chan Kok Gan	University of Malaya
10	Subha Bhassu	University of Malaya
11	Zam Karim	University of Malaya
12	Akmal Adilah Idris	University of Malaya
13	Raha Abd Rahim	Universiti Putra Malaysia
14	Norihan Salleh	Universiti Putra Malaysia
15	Zeti Akhtar Abd Manan	Universiti Malaysia Sarawak
16	Wahap Marni	Universiti Malaysia Sarawak
17	Amin Anak Mangi	Universiti Malaysia Sarawak
18	Mustafa Fadzil	Universiti Sains Malaysia
19	Wan Zaki Wan Mamat	Universiti Sains Malaysia
20	Siti Nasuhah Hamzah	Universiti Sains Malaysia
21	Intan Haslina Ishak	Universiti Sains Malaysia
22	Wong Wan Cheng	Jabatan Pertanian Malaysia
23	Rosmah Jafar	Jabatan Pertanian Sarawak
24	Wee Chien Yong	Malaysian Agricultural Research and Development Institute
25	Norzihan Abdullah	Malaysian Agricultural Research and Development Institute
26	Omar Abd Rasid	Malaysian Palm Oil Board
27	Noor Azmi Shaharuddin	Malaysian Palm Oil Board
28	Maizura Ithnin	Malaysian Palm Oil Board
29	Sunderasan	Lembaga Getah Malaysia
30	Badrul Ezam Badaruddin	Lembaga Getah Malaysia
31	Siti Shuhada Shuib	Lembaga Getah Malaysia
32	Shawn Cheng	Forest Research Institute Malaysia
33	Phon Chooi Khim	Forest Research Institute Malaysia
34	Lokman Hakim	Institute for Medical Research
35	Saraswathy Subramaniam	Institute for Medical Research
36	Lee Han Lim	Institute for Medical Research
37	Nazni Hj Wasi Ahmad	Institute for Medical Research
38	Anza Bt Elias	Institute for Medical Research
39	Noormalin Abdullah	Institute for Medical Research
40	Gan Seng Chiew	Institute for Medical Research
41	Zainah Saat	Institute for Medical Research
42	Norazah Ahmad	Institute for Medical Research
43	Amal Nasir Mustafa	Institute for Medical Research

44	Muhammad Amir Kamaluddin	Institute for Medical Research
45	Naseem Malik	Institute for Medical Research
46	Azizah Mohd Radzi	Institute for Medical Research
47	Chin Yuet Meng	Institute for Medical Research
48	Nur Suffia Sulaiman	Institute for Medical Research
49	Alan Khoo	Institute for Medical Research
50	Rohani Ahmad	Institute for Medical Research
51	Ravindran Thayan	Institute for Medical Research
52	S. Paramaswaran	Institute for Medical Research
53	Lim Kuang Hock	Institute for Medical Research
54	Selvi Subramaniam	Institute for Medical Research
55	Norhaida Hanum Bt Ahmad Tajudin	Institute for Medical Research
56	Chandru A/L Angamuthu	Institute for Medical Research
57	Ummul Haninah Bt Ali	Institute for Medical Research
58	Chew Wai Kien	Universiti Industri Selangor
59	Wan Yusoff	University of Malaya
60	Sumitra S Param	Institute for Medical Research
61	Rusli Hj Tahir	Forestry Department