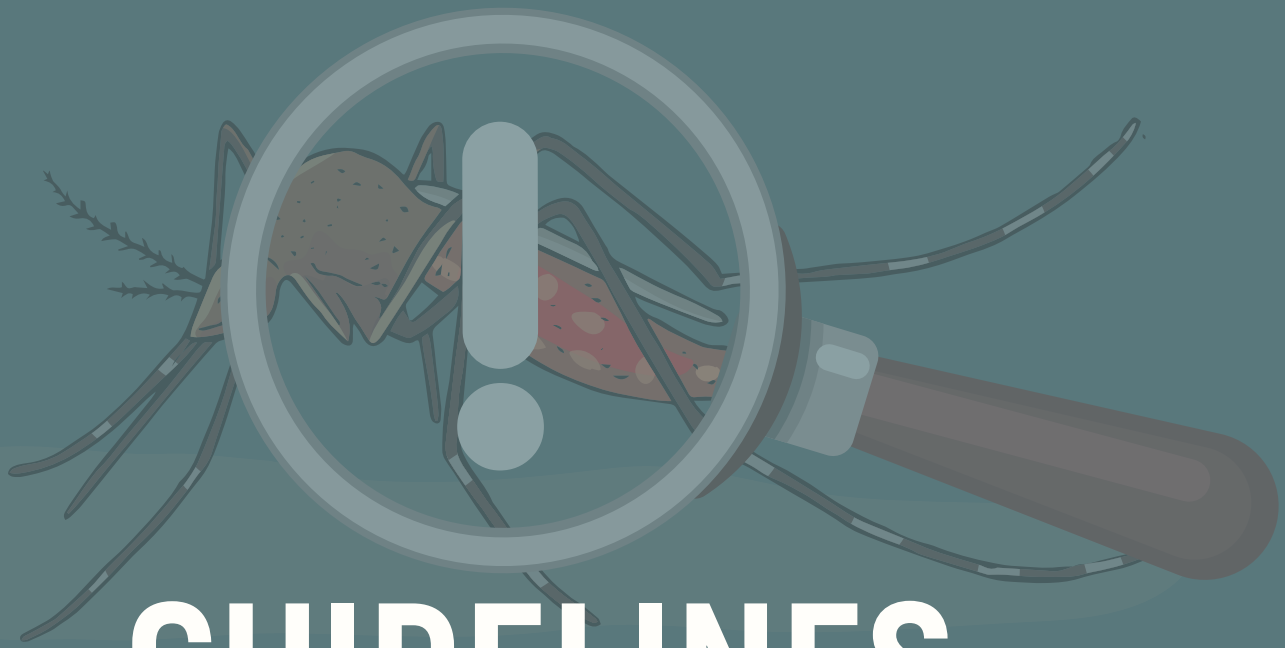

GUIDELINES

FOR TESTING OF GENETICALLY MODIFIED MOSQUITOES IN SRI LANKA

Ministry of Environment, Sri Lanka

2021

Guidelines for testing of genetically modified mosquitoes in Sri Lanka

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1. Introduction

Mosquitoes, a group of small flying insects of over 3,500 species, are vectors of pathogens which cause numerous deadly and/or debilitating diseases such as malaria, dengue, Zika, chikungunya and yellow fever. Malaria, for instance, is caused by *Plasmodium spp.* which is transmitted to humans by the female *Anopheles mosquito*, dengue and chikungunya are caused by the female Aedes aegypti mosquito. These infectious diseases result in millions of deaths every year. Current mosquito control measures rely heavily on the use of chemical insecticides, including insecticide-treated bed nets, indoor and outdoor spraying, and the application of larvicides to stagnant water sources which serve as mosquito breeding sites. Despite extensive application of available control strategies, mosquito-borne diseases continue to pose major global health challenges. The need for better methods to combat mosquito-borne diseases is widely recognized.

Recent research offers the possibility that genetically modified (GM) mosquitoes could be used to prevent pathogen transmission with specificity and the ability to function in areas that are difficult to reach with conventional control methods. Different technologies under consideration include those aimed at reducing the number of mosquito vectors in a given region (population suppression) or rendering the local mosquitoes unable to transmit a pathogen (population replacement). Both types of technologies can be designed in ways so that GM mosquitoes persist for only a brief period of time (self-limiting) or the modification is passed on through local wild mosquitoes and persists indefinitely within the local mosquito population (self-sustaining). Ongoing releases of self-limiting GM mosquitoes will be required to maintain effectiveness. In Sri Lanka, malaria has already been declared eliminated since 2012 whereas extensive efforts are being made for controlling *Aedes* spp. involved in the transmission of the pathogens which cause dengue, chikungunya etc. These efforts include the development of GM mosquitoes for population suppression or population replacement.

Phased testing has been recommended for GM mosquitoes, in which new GM mosquito strategies move from the laboratory, to testing in more natural environments under confined conditions, and finally to open release trials, with each transition dependent upon satisfactory demonstration of efficacy and safety. After a series of consultations over a period of 5 years, the World Health Organization (WHO) developed the "Guidance framework for testing of genetically modified mosquitoes" in 2014[1].

In Sri Lanka, genetically modified organisms (GMOs)/living modified organisms (LMOs)[2] are regulated as per the National Biosafety Framework of Sri Lanka (NBF), 2005. The "Guidelines for testing of genetically modified mosquitoes" in Sri Lanka have been prepared to provide a comprehensive, transparent and science-based framework for Sri Lanka. These Guidelines have been adapted from this Guidance Framework for testing of GM mosquito by WHO with some sections reproduced verbatim. The guidelines are aimed to support regulators in the identification of potential risks that might be caused during the development and testing of GM mosquitoes and guide applicants in planning and conducting safety assessments in support of their activities involving GM mosquitoes in Sri Lanka. More recently developed technologies, such as genome editing and gene drives, which are also being applied to the control of mosquitoes may warrant additional considerations and hence the need for updating the guidelines in the future.

[1] WHO, 2014. Guidance framework for testing of genetically modified mosquitoes. FNIH, WHO, TDR (2014). https://www.who.int/tdr/publications/year/2014/Guidance_framework_mosquitoes.pdf.

[2] LMO is defined as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology; LMOs are considered to be synonymous with genetically modified organisms (GMOs).

The "Guidelines for testing of genetically modified mosquitoes in Sri Lanka" should be used in conjunction with other national guidance documents and careful attention should be paid to ensure that appropriate experimental studies are conducted to address all necessary information and data requirements.

2. Scope

These guidelines apply to:

i. imported and indigenously developed GM mosquitoes that are intended for field trials or release in Sri Lanka, and

ii. all life forms including eggs, larvae, pupae, and adults as well as reproductive cells.

3. Framework for the regulation of GMOs in Sri Lanka

The NBF, 2005 aims to ensure that the risks likely to be caused by modern biotechnology and its products are minimized and biodiversity, human health and environment are protected through the formulation of relevant policies, regulations, technical guidelines and the establishment of management bodies and supervisory mechanisms. Accordingly, GMOs/LMOs undergo a case-by-case risk assessment to evaluate any potential adverse impacts prior to release in Sri Lanka.

In Sri Lanka, activities involving GMOs/LMOs are regulated by the Central Environment Authority under The Ministry of Environment (MoE) as the National Competent Authority (NCA). Relevant government organizations that serve as the Sectoral Competent Authorities (SCAs) include Department of Animal Production and Health, Department of Fisheries and Aquatic Resources, Department of Agriculture, Department of Health Services, and Department of Wildlife Conservation. The SCAs are considered as expert/technical bodies for risk assessment and risk management.

Also, Sri Lanka as a Party to the Cartagena Protocol on Biosafety is obligated as all Parties to make decisions on the import of LMOs for intentional introduction into the environment in accordance with scientifically sound risk assessments. These assessments aim at identifying and evaluating the potential adverse effects· of LMOs. Annex III to the Cartagena Protocol on Biosafety sets forth the principles and methodologies on how to conduct a risk assessment.

Regulatory oversight is required at all stages of testing of GM Mosquitoes in Sri Lanka including laboratory studies and field testing. Guidelines for safe use of GMOs/LMOs in the laboratory provide details for biosafety levels for insects. The activities that can be conducted at different biosafety levels have been explained.

4. Risk Analysis approach

Biosafety considerations for GM mosquitoes address their safe use through the proper assessment of risks to the environment and human health, and the proper management of those risks. Risk is the combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the unwanted consequences will occur. Risk analysis is an objective process to identify what hazards are relevant, how significant the risks are, how they can be managed, and how both the risks and their management can be communicated effectively to all concerned. Risks should be examined and responded to through established protocols within the "Risk Analysis Framework" determined by Sri Lankan biosafety regulations on environmental and human health risks, and their acceptance or management.

The purpose of risk assessment of GM mosquitoes is to identify and evaluate risks to the health and safety of people and the environment from the release of GM mosquitoes, when compared with the non-GM version of the mosquitoes and to characterize the risks on the basis of severity and likelihood.

Risk assessment and risk management must be focused on the particular GM mosquito application under examination. Specific risk assessment and risk management considerations will vary among various GM mosquito technologies and in different phases of testing. Regulatory decision-making also includes opportunities for public consultation prior to release.

An important concept of risk analysis is that while an event theoretically may occur, it will not necessarily be harmful, because either it does not have a perceived negative effect or it does not have an effect specified as harmful in regulations. Risk analysis must be undertaken on a case-by-case basis to identify and manage any adverse effects to the environment and/or human health.

5. Key Considerations for testing of GM mosquitoes

5.1 Phased Testing

A phased testing pathway is recommended for GM mosquitoes, with systematic assessment of safety and efficacy at each step. These include:

Phase 1 – Laboratory/cage studies,
Phase 2 – Confined field trials,
Phase 3 – Stage open field releases, and
Phase 4 – Post implementation surveillance.

The following illustration describes the above as a unidirectional pathway. However, in practice, repetitions of some segments of the pathway may be required in order to improve the technology and refine the procedure until the requirements for moving to the next phase are met (Fig. 1).

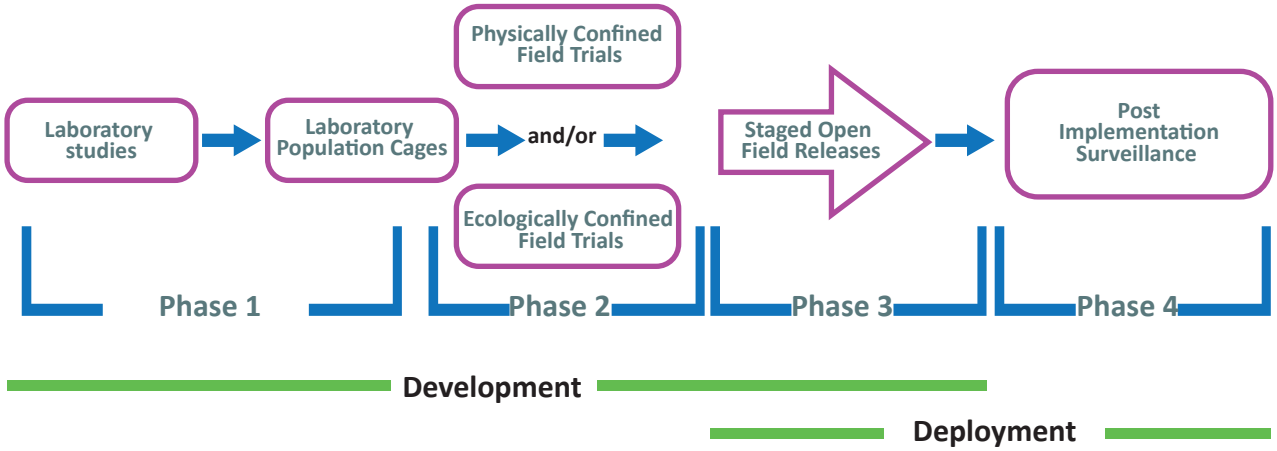

Fig.1. The schematic diagram illustrating the unidirectional pathway activities pertaining to development of basic data and deployment of a GMO in the field

The specific experimental designs to be used may vary widely. According to the specific mosquitoes, study sites for testing and progression of experiments from the laboratory to the field will require reconsideration at each stage.

5.2 Site Characteristics

Baseline information on key site characteristics is important to ensure that field trials can be adequately planned and interpreted. Selection criteria might include the distribution of principal vectors in the release area, the location of mosquito larval sites, climatic conditions, knowledge of active transmission (if any) of the target disease pathogen at the site, geographical isolation of the site for confined trials so that there is negligible chance of any impact outside the trial area, existing data on the transmission dynamics of the target disease, and existing surveillance and control systems for both vectors and disease, the likelihood of obtaining regulatory, social and political approval for research on GMMs in the study community and surrounding areas, and the ability to continue existing vector control practices.

5.3 Appropriate Comparators

The choice of non-modified mosquito comparators is essential in risk assessment of GM mosquitoes. In some phases, such as in Phase I, the ancestral laboratory line from which the transgenic mosquito line was derived, is a logical comparator. Sometimes, ancestral laboratory lines may lead to a less precise risk assessment relevant to the characterization of the genetic modification compared to wild population due to the loss of fitness because of intensive rearing in the laboratory. In cases when it is proposed to use alternative non-modified comparators (such as field-derived strains of the modified species), it will require careful scrutiny of the genetic background together with physiological and behavioural characteristics. Such comparators may be more appropriate for field comparisons in later stages.

It is likely that GM mosquitoes will be used in the absence of other control methods in Phase 1 and large outdoor cage testing of Phase 2. Conventional experimental approaches involving direct comparison between GM mosquito cages and control cages with random treatment assignment may be used. In such cases, only entomological measurements can be made. A sufficient number of replicates should be used to detect the expected difference in the entomological outcomes between GM mosquito and control cages. At later stages, the novel mosquito control system incorporating GMMs may be compared with conventional control system. The comparison is related to the scale and purpose at this phase and addresses the risks arising across the integrated systems of control.

5.4 Efficacy evaluation

Efficacy measurements of GM mosquitoes can be defined by entomological and epidemiological outcomes. These differ according to the disease, the vector species and the epidemiological circumstances. Efficacy measurements will also vary depending on the intended effects of GM mosquito strategies and testing phases. For example, transmission intensity cannot be measured in Phase 1 testing in a small-scale laboratory setting or in larger population cages. Instead, estimations of transgene phenotype stability, population reduction, and transgene spread and frequency are feasible, and are meaningful indicators of GM efficacy. These must be considered within the context of the disease transmission setting in which the GM mosquitoes will be tested and/or deployed. Entomological outcomes must be monitored throughout the phases of development. As testing moves to settings in which humans are, or may be, present, increased attention to epidemiological outcomes must be added.

5.5 Mathematical modelling

RA can be enhanced by coupling experiments and/or observations with mathematical modelling. Mathematical modelling can highlight the range of parameters necessary for RA. The overall aim of mathematical modelling within the RA context of risk assessment can be undertaken on case-by -case basis to predict behaviour based on properties and assumptions of transgenic modifications and assessing the likelihood of events. For example, a specific set of molecular modifications, mathematical models might be used to predict whether or not the fitness of the GM mosquito will be enhanced by the molecular modification. Mathematical modelling of inter-specific interactions might also be useful to reveal potential structural alteration to the ecological (biotic) effects. Under self-sustaining strategies, assessing whether the heritable modification will have an impact on the ecological competitive ability of the GMM and/or ecological interactions could be accomplishes using data from small-scale semi-artificial population trials in the laboratory.

5.6. ‘Go’ and ‘no-go’ decision criteria

Transition from the laboratory to the field or from one phase to the next will be subject ‘go’/ ‘no-go’ decision criteria, including efficacy and safety endpoints, regulatory and ethical approval and social acceptance. There is always a need to plan clear stated performance milestones at which point the project either proceeds to the next level or to modify or the trial is discontinued. The consequences of the trials become greater from physically confined to ecologically confined and open-field release. Continuous monitoring to identify the adverse effects must increase accordingly.

6. Phase 1 testing: Laboratory/cages

Phase 1 studies include small scale laboratory studies for efficacy and safety testing, followed by testing in larger population cages in laboratory setting under appropriate containment facilities and procedures. Guidelines for the safe use of GMOs/LMOs in the laboratory may be consulted appropriate in conjunction with international guidance on arthropod containment levels, e.g., American Committee of Medical Entomology[3], which are regularly updated from time to time. Phase 1 testing is at an early stage of development, there will inevitably be limited information on the stability and effect of genetic modifications and a cautious approach is essential, primarily due to uncertainty rather than any established hazard. Risk assessment in preparation for Phase 1 will include conditions under which laboratory studies can be conducted, including the acceptable level of exposure to GM mosquitoes by research personnel, acceptable security measures to prevent GM mosquitoes from escaping, and appropriate methods for disposing of waste materials should be followed. Emergency plan for control or mitigation measures should be in place to eliminate escaped GM mosquitoes through proven means, such as pesticide applications, insect trapping etc. Phase 1 trials allow preliminary assessment of whether GM mosquitoes demonstrate the desired biological and functional characteristics, with an eye toward future efficacy and safety.

Only entomological outcomes can be determined in Phase 1. Pathogen interactions can, also, be measured. Typical studies/information in Phase I that can be undertaken, are as follows:

- Basic description of the transgene, including its sequence, insertion site, phenotype and inheritance. This information will be used during Phases 2 and 3 to confirm the GM mosquito's characteristics.
- Stability of the transgene and its phenotype.
- Life-history characteristics in controlled environments.
- Mating competitiveness against laboratory mosquito strains.
- Frequency of GM mosquitoes that express the desired characteristic and the level of expression.
- Capability to host and transmit pathogen isolates.
- For population suppression strategies, rate of suppression in laboratory cage trials.
- Mating frequencies and egg hatching rates within the strain and in crosses to laboratory strains.
- GM mosquito release simulations in large indoor cages.
- Modelling effects anticipated in wild populations.
- Establishment of Standard Operating Procedures (SOPs) for GM mosquito production and release.

[3] https://safety.fsu.edu/safety_manual/supporting_docs/Arthropod%20Containment%20Guidelines.pdf

7. Phase 2 testing: Field testing

Phase 2 testing involves confined testing in a more natural setting but conditions that will limit release into the environment. Testing in Phase 2 may be undertaken in physical confinement (sometimes termed as containment) and/or small scale ecologically confined field release as indicated below.

(i) **Physically confined or contained:** This refers to trials performed in large outdoor cages that simulate the disease endemic setting but minimizing the possibility of escape. The escape is highly unlikely due to physical barriers and special procedures. Such trials allow rapid termination and simple detection of escapees.

(ii) **Ecologically confined:** This refers to small scale ecologically confined field release under geographic/spatial and/or climatic isolation intended to limit the spread of GM mosquitoes into the environment. These trials are conducted in delimited areas from which escape is unlikely due to some ecological or geographical isolating factor. These include ecological or physical islands.

The decision about the requirement of one or both components of Phase 2 testing will depend on the nature of GM mosquito technology, prior knowledge of its effects in other environments, taking into account the process of risk assessment. Regulators will determine the request of types of trials more by safety rather than by efficacy considerations. For some GM mosquito technologies, physical confinement is not a necessary step in the testing pathway, instead the genetic or ecological confinement conditions may provide sufficient risk reduction. Particularly, in cases where Phase 1 results have demonstrated that there is limited potential for dispersal, physical confinement may be less important. For example, for trials where the GM mosquito's progeny does not mature to adults, or where the GM mosquito is not expected to persist. Previous evidence from laboratory or other confined trials may demonstrate that protocols to discriminate the sex of the released mosquitoes, and their phenotypic properties, are sufficient to ensure safety in an ecologically confined trial. Conversely, for gene drive constructs designed to be inherited at greater than Mendelian frequencies, and thus spread through populations, physical confinement may be required throughout development.

Regulatory requirements will differ for physically confined versus ecologically confined trials, since ecologically confined field trials involve intentional, although limited, release into the environment. In physically confined field trials, particular attention should be paid to cage designs and local environmental conditions at the chosen field site. Aspects of local geological, ecological and regulatory criteria will underpin the design of physically confined field cages and trial implementation. Ecologically confined field trials may take place in locations that do not favour the long-term survival of the GM mosquitoes, or in ecologically isolated locations (such as an area surrounded by water, deserts or mountains). Combinations of physically and ecologically confined trials are possible.

Risk management measures including restricted access, clear and well managed standard operating procedures (SOPs) should be used to mitigate risks associated with confined field testing. Applicants are required to put in place SOPs to document how transgenic material should be moved from laboratory to the field prior to release, protocols for ensuring site security/cage suitability, criteria for release strategies, surveillance during the trials and post-trial removal of material and cages. Monitoring the performance of containment/ confinement measures will minimize risk from unintended release. Periodic sampling of the GM mosquito population in the trial should be undertaken to determine the stability of the transgenes and any recognizable change in the genetics of the population that may affect the impact of the technology. Plans would need to indicate how residual populations in cages would be eliminated after a trial; in the case that the risk is determined to be negligible, this might simply involve allowing the material to enter the decomposer food chain. However, if such residual material were identified to constitute a hazard, more aggressive risk management of residual dead material would need to be considered.

Understanding the risk associated with a breach of physical/ecological confinement requires appropriate consideration. A breach of physical confinement may lead to the loss of GM mosquitoes or loss of genetic material into the wider receiving environment. The risk assessment should take into account cage designs, experimental planning, emergency preparation, training, and site security. A mechanism for practical and reliable discrimination of GM mosquitoes and wild mosquitoes should be available prior to initiating Phase 2 experiments (for example, through the use of fluorescent dyes or dusts and/or phenotypic or genetic markers). Where release of male only GM mosquitoes is part of the system, methods for reliable sex-selection prior to release will be necessary to ensure an acceptable sex ratio is achieved. Other biological considerations for risk assessment in preparation for Phase 2 testing would include what is known about the local dispersal and gene flow patterns for target mosquitoes and what pathogens they transmit in the receiving environment.

In terms of data to be collected the focus of Phase 2 trials is to continue assessment of biological and functional activity of GM mosquitoes, including their effect on local/wild type mosquitoes. Because of the limited scale, the information on the disease impact would need to be studied in larger GM mosquito trials, taking into account the regulatory and ethical considerations. This would require basic ecological, entomological and epidemiological information so as to identify clear end points. Phase 2 trials should be structured to provide relevant information on the ecological processes critical to the evaluation, efficacy and success of the GM mosquitoes. Additional considerations for biological information to be collected in Phase 2 testing will relate to the specific GM mosquito approach under consideration. Epidemiological outcomes may begin to be measured in confined release trials, although, for the reasons explained above, this will be uncommon due to the small scale of the trials.

Entomological activities in physical confinement

- Mating competitiveness against mosquito strains having a wild genetic constitution.
- Frequency of GM mosquitoes that express the desired characteristic and the level of expression in strains containing wild genetic background.
- Capability of GM mosquitoes containing local wild genetic constitution to host and transmit local pathogen isolates.
- For population suppression strategies, the rate of suppression against wild mosquitoes in cage trials.
- Egg hatching rates in crosses to wild mosquitoes.
- GM mosquito release simulations in large outdoor cages.

Entomological activities in ecological confinement

- Establishment of go and no-go criteria.
- Compatibility studies with other mosquito control measures.
- Measures of GM mosquito dispersal.
- Baseline studies of vector composition and abundance.
- Measures of transgene functionality and mutation rate.
- For population suppression strategies, the rate of suppression against wild mosquitoes.
- Randomized treatments of similar trial sites.
- Model refinement based on Phase 2 entomology and epidemiology observations; estimation of impact on entomological inoculation rate (EIR).
- For population suppression strategies, refined measures of relationship between sterility and population suppression.

Epidemiological activities in ecological confinement

- Measures of the ability to sustain development of local pathogen isolates as an indication of potential for transmission.

8. Phase 3 testing: Staged open field releases

Based on satisfactory results of confined testing in Phase 2, the GM mosquito technology may proceed to staged open release trials under Phase 3. This will involve a series of sequential trials of increasing size, duration and complexity, to be conducted at a single site or multiple sites. These trials may be designed to assess performance under various conditions, such as different levels of pathogen transmission, seasonal variations in mosquito density, or presence of other disease vectors in the region. While the measurement of entomological parameters is likely to remain the focus of early Phase 3 trials, later trials in this phase may include measurement of the impact of GM mosquitoes on infection and/or disease in human populations. Trials to show epidemiological impact must be designed accordingly, with considerable thought on the needs for achieving a statistically meaningful result. Although still focused on intense examination of the function and efficacy of GM mosquitoes, Phase 3 trials effectively institute a limited deployment of the technology; this will especially be the case for self-sustaining approaches that are anticipated to persist.

Site selection for open releases should consider the isolation of the site, the structure and knowledge of the vector population, the disease dynamics and the implications of any differential impacts among local communities. It should also consider the size of the open-field release site, which will dictate the site characteristics. When selecting the site, risk assessment could make use of the substantial advances in technology and knowledge of geographical surveys (e.g., global positioning systems, geographical information systems and high-resolution satellite images), and predictive models of habitat suitability. These methodological advances allow the thorough analysis of temporally and spatially referenced data relevant to both mosquito ecology and disease burden. Choice of appropriate site size and layout will enhance both the biological and statistical validity of the open-field release. Cluster size and number should be predicated on the focused aims and endpoints of the staged open release. Plans for open field releases to assess efficacy of spread (e.g., competitiveness, longevity, dispersal) should consider the need for well-designed and replicated experiments at a spatial scale that limits the effects of immigration and other spatially dynamic processes. Similarly, RA and RM for open releases designed to demonstrate suppression or replacement potential should consider the measurable parameters (such as population density or the proportion of a genotype in the field population) needed to demonstrate conclusively the aim of the release. If the end points are focused on disease control then appropriate knowledge of the size of the human population, level of disease burden and ethical issues related to testing of disease interventions should be incorporated into the RA. The evaluation of GM mosquito effects on the incidence of the target infection will be part of efficacy testing, but based on studies of vector capacity in phases 1 and 2, consideration should be given to the need for monitoring other vector-borne diseases. Assessing the different types of release strategy for both self-limiting and self-sustaining approaches is important, as knowledge of the connectivity between the population within the target zone and the surrounding populations is important in preventing any adverse increase in the entomological or epidemiological burden associated with the target mosquito.

Risk management in Phase 3 will be similar to Phase 2 above but will need to be expanded in scale to account for the lack of confinement. The evaluation of surveillance data would benefit from the availability of appropriate baselines before release (such as the level and seasonal pattern of disease burden, the past levels of the vector population, effects of conventional vector-control methods). A recall or control plan of sufficient scale to limit spread should be agreed upon and be available before field release, if there is ongoing concern about risk. There should be a procedure to monitor any degradation of efficacy in the GM mosquito control system that may indicate that resistance to the effector has developed. The degree of resistance, its rate of increase and possible attendant hazards must be evaluated. Regular sampling of wild populations should be considered as a method to detect resistance.

Most species of mosquitoes normally remain within a few hundred metres over their life, unless transported by man or strong winds. In Sri Lanka, management should be put in place to avoid and detect movement through transport, in case neighbouring countries have not approved release for testing. Released GM mosquitoes should carry markers that ensure discrimination from wild mosquitoes. In small trials, a treated barrier area downwind may reduce the chance of successful movement towards a border. Staff working on field testing sites should be trained about the risks of moving living specimens and should observe transport protocols when moving any material. Post-trial monitoring should take into account the numbers of GM mosquitoes released, with the aim of achieving an appropriate level of sampling efficiency.

Open testing in Phase 3 will introduce opportunities to gather data on potential hazards in the risk analysis where these data can only be acquired under more natural conditions. It also provides an opportunity to evaluate the performance of GM mosquitoes integrated within complementary conventional control actions. However, considerations of environmental variability, reduced control of experimental variables, and the impact of these on proper experimental design and statistical power are even more influential at this stage. Risk assessment under field trials may provide information on whether the transgenic modification has any chance to increase vectorial capacity (the efficiency of vector-borne disease transmission) or vector competence (the capability of a vector to support the development of a pathogen) under particular circumstances. Assessment of wild-type mosquito population size and dynamics is essential for both self-limiting and self-sustaining approaches. Mark-release-recapture measurements of wild-type mosquitoes can provide a baseline for assessing the necessary release ratio and the risks associated with releasing large numbers of transgenic mosquitoes. Assessment of population size, age structure and/or sex ratio post release should take into account sufficient time for a new equilibrium to be established. The fitness of a population should be assessed to determine if there is a risk of population increase in the longer term. At the end of Phase 3, the GM mosquitoes stand on the verge of routine use as a public health intervention. Therefore, sufficient data should be collected to understand the effect of the GM mosquitoes on disease transmission, ecological interactions and the spatial characteristics of dispersal and transgene persistence. This will involve extensive post-release monitoring of wild populations for the transgene, widespread assays of the GM mosquitoes for phenotypic and genetic marker stability, and an assessment of the performance of the RA and RM strategies. These considerations will constitute an important part of any decision to move forward with deployment, a decision that will necessarily also take into account broader cost-benefit, acceptance and national public health goals.

Phase 3 is likely to begin with limited releases intended to understand the delivery requirements and functionality of GM mosquitoes under different circumstances, such as different ecologies, mosquito demographics and seasons. Large trials to determine epidemiological impact should only be planned after this information is at hand, as it will be necessary for trial design and interpretation. It is recommended that randomized cluster trials be included in the design for late Phase 3.

Entomological activities

- Compatibility studies with other mosquito control measures.
- Direct measures of EIR when possible.
- Baseline studies of vector composition and abundance.
- Measures of transgene functionality, phenotypic stability and mutation rate.
- Measures of GM mosquito dispersal.
- For population suppression strategies, the rate of suppression of wild populations.
- Model refinement and validation based on Phase 2 entomological and epidemiological observations.
- For refractory GM mosquitoes, measures of native pathogen development and transmission in progeny from natural mating of the GM mosquitoes to wild mosquitoes.
- Methods for measuring or estimating GM mosquito frequency and cross-species gene transfer and consideration of how long these activities should continue.

Epidemiological activities

- Disease incidence/prevalence studies during intervention trials.
- Post-treatment active and/or passive disease incidence/prevalence, and consideration of how long these activities should continue.

9. Phase 4 testing: Post implementation surveillance

Results of Phase 3 testing will form the basis for determination as to whether the technology should move into wider scale application as part of a national or regional programme for vector and disease control. The ultimate decision on deployment of GM mosquitoes as a public health tool will be taken with the involvement of authorities responsible for determining national or regional disease control priorities. Risk assessment for Phase 4 will also take into account whether any specific surveillance plan need to be put in place for ongoing monitoring of GM mosquito effects. This would also include predicting the likely manifestation of any potential resistance. In the context of implementation, risk evaluation will be set against the benefits of GM mosquitoes in improving human health. The results of risk analysis will be taken into account to make a decision about whether and how to allow large scale GM mosquito deployment in Sri Lanka and whether to adopt GM mosquitoes as a component of National Disease Control Programme.

Phase 4 constitutes an ongoing surveillance phase that will assess effectiveness under operational conditions (both entomological and epidemiological impacts), accompanied by monitoring of safety over time and under diverse situations. Long term surveillance of safety for human health will be analogous to the pharmacovigilance applied in medicine but, in the case of GM mosquitoes, aspects of environmental safety should also be considered. Ongoing monitoring will be aimed at ensuring sustained quality and performance for disease control, and determining whether any changes are needed in management of either the GM mosquito technology itself or other aspects of an integrated control programme.

If required, a surveillance plan may be designed and implemented to detect movement and introgression of the genetic construct within vector populations and detect unintended changes in vector biology that may result in changes in biological fitness, adverse changes in vectorial capacity, and changes in nuisance impacts. In case of failure to perform as expected or required, emergency control or mitigation measures need to be available to eliminate escaped and established GM mosquitoes. Like any public health intervention, GM mosquitoes will require ongoing monitoring to determine whether their efficacy has diminished with time or because of unexpected effects that become evident when used in new areas. Appropriate measurement of the entomological outcomes that guided deployment of the GM mosquito must be continued after the trials cease. Depending on the type of GM mosquito technology and the deployment strategy, multi-year follow-up may be required.

GM mosquitoes that reach Phase 4 will have undergone extensive efficacy testing. Their behaviour in natural settings will be established by Phase 3 activities. However, it cannot be assumed that they will continue to behave as expected. By analogy with the implementation of insecticides used for long- lasting insecticide treated bed nets (LLIN), indoor residual spraying (IRS) and larviciding, efficacy can change due to changes in the genetic constitution of the mosquitoes or external factors such as weather and human activities. However, the intervention at this point is no longer experimental, but is a control measure whose ongoing effectiveness in a public health programme is being determined.

A subset of the epidemiological outcomes that were utilized during Phase 3 trials should be monitored in order to determine whether the positive effects on human populations are being sustained. It is likely that if the GM mosquitoes were deployed over large areas, only longitudinal passive clinical case surveillance would be practical. In case a loss of efficacy is noticed – similar to the appearance of insecticide resistance with conventional control – any second-generation GM mosquitoes that may be created must also be tested in phases 1–3, and monitored in Phase 4.

Entomological activities

- Direct measures of EIR under novel conditions (when possible).
- Widespread intermittent sampling of transgene functionality and mutation rate.
- Wide-scale intermittent measurement of GM mosquito dispersal and gene flow.
- For population suppression strategies, sampling of the degree of suppression of wild populations.
- Model refinement based on entomological and epidemiological observations.
- For refractory GM mosquitoes, observation of native pathogen development in mosquitoes collected in disparate settings.

Epidemiological activities

- Longitudinal passive case detection of targeted disease and other mosquito-borne diseases.

The data requirements for each species/trait combination will be different on a case by case basis. An indicative list of information required in support of risk assessment for field trials/ environmental release of GM mosquitoes is given in Annex-1.

10. Public engagement

The critical path for GM mosquito development will include not only proof of efficacy, but also proof of acceptability and deliverability. Activities for engagement of stakeholders need to be considered broadly at three levels, viz. project level, community engagement and general public. At the initial stages i.e. project level, the focus of engagement is limited to the project team, interactions with advisory committee and consultants as well as other scientist on case-by-case basis. Once the field trial sites are identified, deliberations may be held with interested groups in respective sites. Community engagement is required with people living within a proximity of the trial site. Individuals not immediately associated with the trial site such as public health or development organizations, civil society organizations, press and general public may be interested in the conduct and outcome of research and need to be informed about project goals and activities.

Adequate plans for communication and engagement should be put in place before the earliest stages of field testing. Community engagement and authorization activities will be necessary particularly in Phase 2 of the GMO testing pathway and will expand in Phase 3. The need for public engagement activities is likely to continue in Phase 4.

Glossary of Terms

Community engagement: practices undertaken to inform stakeholders about the diseases and vectors of interest and goals of a proposed research study or intervention trial, and to understand their perspectives and reaction.

Confinement: Utilization of measures that seek to prevent unplanned or uncontrolled release of organisms into the environment. This may involve physical confinement (sometimes termed "containment") within a large cage that simulates the disease-endemic setting while minimizing the possibility of escape and/or ecological confinement by geographic/spatial and/or climatic isolation.

Endpoint: an event or outcome that can be measured objectively to determine whether the intervention being studied has the desired effect.

Entomological inoculation rate (EIR): a measure of the degree of infection risk that a human population is exposed to for a particular disease, as determined by assessing the vector mosquito population. It is described by the frequency of infectious mosquito feeding upon a person within some unit of time, such as per day or year.

Genetically modified mosquitoes (GM mosquitoes): Also called genetically engineered, transgenic, or living modified mosquitoes that have heritable traits derived through use of recombinant DNA technology, which alter the strain, line, or colony in a manner usually intended to result in reduction of the transmission of mosquito-borne human diseases.

Living modified organism (LMO): Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology; LMOs are considered to be synonymous with genetically modified organisms (GMOs)

Modern biotechnology: means the application of:

(i) In vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles, or

(ii) Fusion of cells beyond the taxonomic family, that overcome natural and physiological, reproductive or recombinant barriers, and that are not the techniques used in traditional breeding and selection.

Pathogen: An organism that causes disease. For example, in dengue infection, the pathogen is a virus. In malaria infection, the pathogen is a unicellular parasite.

Population replacement: Strategies that target vector competence with the intent to reduce the inherent ability of individual mosquitoes to transmit a given pathogen.

Population suppression: Strategies that target vector "demography" with the intent to reduce (suppress) the size of the natural mosquito population to the extent that it would not be able to sustain pathogen transmission.

Self-limiting: GM mosquito approaches where the genetic modification will not pass on indefinitely through subsequent generations.

Self-sustaining (also called self-propagating): GM mosquito approaches where the heritable modification is spread and maintained indefinitely through the target population.

Wild: refers here to a colony of mosquitoes isolated recently from the target population or a sample actually collected from natural populations and used without colonization. Such colonies are genetically more similar to natural mosquitoes than highly inbred laboratory strains.

REFERENCES

1. **NAPPO, 2007. Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries, NAPPO Regional Standards for Phytosanitary Measures, North American Plant Protection Organization.**
   https://www.nappo.org/files/1814/3753/9399/RSPM27-e.pdf

2. **ASTMH, 2001. American Society of Tropical Medicine and Hygiene. Arthropod Containment Guidelines, Version 3.1.**
   www.astmh.org/SIC/files/acgv31.pdf.

3. **WHO, 2014. Guidance framework for testing of genetically modified mosquitoes. FNIH, WHO, TDR (2014).**
   https://www.who.int/tdr/publications/year/2014/Guidance_framework_mosquitoes.pdf.

Annex-I

INFORMATION REQUIRMENTS TOWARDS APPLYING FOR FIELD TRIALS/ ENVIRONMENTAL RELEASE OF A GENETICALLY MODIFIED MOSQUITO

CHECKLIST OF INFORMATION TO BE SUBMITTED IN SUPPORT OF RISK ASSESSMENT

The below checklists are intended to provide useful reference to both applicants and risk assessors. Decisions about what information is required for any particular risk assessment will be made on a case by case basis. Information listed here may not be required in all cases, and information not listed here may be required for a particular case if additional information needs are identified.

1. Description of the GM mosquito

Information provided	YES	NO
Name of the GM mosquito		
Common name of the mosquito		
Scientific name of the mosquito		
Description of the introduced trait		
Origin or source of the introduced genes		
Unique Identifier (if applicable)		
Intended Use (e.g., field trial/ release)		
Purpose of the genetic modification		
Life history parameters		
Life stage to be released		
Information on specific laboratory line or colony of the GM mosquito		
Pedigree map of the GM mosquito (providing information about the number of generations rearing colonies)		
Geographical areas within Sri Lanka to which distribution is intended		
History of use in control programmes		
Methods to distinguish the modified mosquito from non-modified mosquito* (molecular, morphological or other methods)		
Additional Details, if imported		
Source of GM Mosquitoes (Details of the institution/agency and contact person)		
Specifications and quantity of imported material		
Status of approval in country of origin		
Status of trial/use in other countries, if any		

2. Description of the Non-Modified mosquito

Information Provided	YES	NO
Taxonomy		
Phenotypic characteristics		
Reproduction (generation time, mode of reproduction, sexual compatibility etc.)		
Means and extent of dissemination		
Survivability		
Natural habitat and range		

*The original strain used for modification

For any information not included, please provide a rationale as to why the information is not relevant or necessary for risk assessment of the GM mosquitoes, or what information is being provided in its place.

3. Description of the Donor Organism

This information should be provided for the donor of each transgene present in the GM mosquito

Information Provided	YES	NO
Common name		
Scientific name		
Taxonomic classification		
Size of the genetic material		
Size of the gene inserted into the recipient		
Intended function of the gene(s) introduced in to the recipient.		
Genetic components from donor if present in any other GMO/LMO authorised field trials or release in Sri Lanka or other countries.		

For any information not included, please provide a rationale as to why the information is not relevant or necessary for risk assessment of the GM mosquitoes, or what information is being provided in its place.

4. Description of the genetic modification

Information provided	YES	NO
Modification method		
Characterisation of the genetic material		
Details of modifications		
Summary diagram of the genetic components		

For any information not included, please provide a rationale as to why the information is not relevant or necessary for risk assessment of the GM mosquitoes, or what information is being provided in its place.

5. Molecular Characterization of Transgene(s)

The following information should be provided for each transgene in the GM mosquito

Information provided	YES	NO
Genetic modification		
Characterization and description of the inserted genetic material		
Number of insertion sites		
Description of the organization of the genetic material at each insertion site		
Sequence data of the inserted material and flanking regions		
Identification of open reading frames within the inserted DNA or contiguous genome of mosquito		
Expressed substances		
Gene product (e.g. protein or RNA)		
Function of the gene product		
Phenotypic description of the new trait		
The level and site of expression of the gene product in GM mosquitoes		
Confirmation of intended effects		
Evidence supporting the function of any modifications to the amino acid sequence or post translational modification		
Evidence of stable inheritance		

For any information not included, please provide a rationale as to why the information is not relevant or necessary for risk assessment of the GM mosquitoes, or what information is being provided in its place.

6. Phenotypic Characteristics of the GM mosquito

Information provided	YES	NO
Information about phenotypic stability of the inserted trait		
Life history parameters (e.g. timing and duration of reproduction, survivorship of each life stage, longevity etc.))		
Mating strategy		
Flight ability		
Oviposition rate		
Ability to persist in the environment.		
Response to specific biotic and abiotic stresses relative to responses of the unmodified mosquitoes.		
Response to detection survey tools, e.g., traps		
Status of susceptibility to labelled insecticide(s) employed as a risk management option or for control of the wild type mosquito.		

For any information not included, please provide a rationale as to why the information is not relevant or necessary for risk assessment of the GM mosquitoes, or what information is being provided in its place.

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7. Description of confined field/ release site

Information provided	YES	NO
Location		
No. of sites		
A map of the site, buffer zones, and relevant adjacent areas (Global Positioning System coordinates should be included)		
Proximity to populations of the same species as the GM mosquito and closely related species.		
Proximity to sensitive or protected ecological areas.		
Protocols for surveillance for the presence of GM mosquitoes		
Population size		
Spatial distribution		
Vectorial capacity		
Behavioural resistance		
Biochemical resistance		

For any information not included, please provide a rationale as to why the information is not relevant or necessary for risk assessment of the GM mosquitoes, or what information is being provided in its place.

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8. Description of the confinement measures followed during field trials

Information provided	YES	NO
Physical confinement (includes details of physical security, access controls, personal protective equipment, and other security measures.)		
Biological confinement (biological confinement measures used and data should be provided demonstrating the efficacy of these measures, e.g., the efficacy of genetic or irradiation-induced sterility)		
Geographic isolation, if any		

Please provide the description of the measures to be followed.

9. Risk Management Plan

This risk management plan should consider and have procedural actions for the following elements

Information provided	YES	NO
Methods for monitoring and detection		
Methods and procedures for controlling the GMO in case of unexpected spread or to “clean up the affected area”; this could include such as extended trapping, or the use of insecticides.		
Plans for protecting human health and the environment in the case of an adverse event occurring		

Please provide the description of the measures to be followed.

10. Any other information s stipulated by regulatory authorities.

National Focal Point and Coordinating Agency for Biosafety in accordance with the Cartagena Protocol in Sri Lanka

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