AUDA-NEPAD
AFRICAN UNION DEVELOPMENT AGENCY

Guidelines for Risk Analysis for the Testing and Deployment of Genetically Modified Mosquitoes

West Africa Integrated Vector Management Programme

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About The AU, AUDA-NEPAD and WAHO

The African Union (AU)

The African Union (AU) is a body of 55 member states that make up the countries of the African Continent. It was officially launched in 2002 as a successor to the Organization of African Unity (OAU), which ran from 1963 to1999. The decision to re-launch Africa's pan-African organisation was the outcome of a consensus by African leaders that in order to realise Africa's potential, there was a need to re-focus attention from the fight for decolonisation and ridding the continent of apartheid hitherto pursued under the OAU, towards increased cooperation and integration of African states to drive Africa's growth and economic development. The AU is guided by its vision of *An integrated, prosperous and peaceful Africa, driven by its own citizens and representing a dynamic force in the global arena [1].*

To realise this vision, the Africa Union developed and adopted a 50-year strategic plan called Agenda 2063 [2]. Agenda 2063 is the continent's strategic framework that aims to deliver on its goal for inclusive and sustainable development and is a concrete manifestation of the pan-African drive for unity, self-determination, freedom, progress and collective prosperity pursued under Pan-Africanism and African Renaissance.

The AU has been steadfast in proposing more enabling and science-based approaches to the challenges of the continent. Its report on gene drives clearly embraces the technology as a realistic option for effective disease control. A constructive development along this path was witnessed at the 29th Ordinary Session of Heads of State and Government of the African Union in Addis Ababa, where pursuant to Decision *Assembly/AU/Dec.649 (XXIX),* the session embraced the gene drive technology as a realistic option for malaria control. The session, in its decision, requested the African Union Commission (AUC), West African Health Organization (WAHO) and African Union Development Agency-New Partnership for Africa's Development (AUDA-NEPAD) to collectively support the initiative [3].

In 2018, through recommendations of the African ministers responsible for science and technology *EX.CL/Dec. 987(XXXII),* the Executive Council of the African Union encouraged member states to harness emerging technologies, including gene drive, in their development initiatives [4].

The decisions above have offered solid policy statements for the continent regarding gene drives for human health purposes, which have impacted discussions in AU member states. It is a basis for a harmonised approach for Africa in the development of policy regulations and guidelines such as this to facilitate the responsible and safe application of the technologies for research and subsequent deployment.

The African Union Development Agency - NEPAD (AUDA-NEPAD)

At the 31st Ordinary Session of the Assembly of African Union Heads of State and Government held in Nouakchott, Mauritania from 25th June to 2nd July 2018, the Heads of State and Government approved the transformation of the New Partnership for Africa's Development (NEPAD) Planning and Coordinating Agency into the African Union Development Agency (AUDA) as the technical body of the African Union with its own legal identity, defined by its own statute [6]. The objectives of AUDA-NEPAD are to: a) coordinate and execute priority regional and continental projects to promote regional integration towards the accelerated realisation of Agenda 2063; b) strengthen capacity of African Union Member States and regional bodies; c) advance knowledge-based advisory support; d) undertake the full range of resource mobilisation; and e) serve as the continent's technical interface with all Africa's development stakeholders and development partners.

The West African Health Organization (WAHO)

The West African Health Organization (WAHO) was established in 1987 when the Heads of State and Government from all fifteen countries in the Economic Community of West African States (ECOWAS) adopted and thereafter ratified the protocol for its creation. WAHO has transcended linguistic borders and hurdles in the sub-region to serve all fifteen ECOWAS Member States. The protocol grants WAHO the status of a specialised agency of ECOWAS and, as guided by its mission statement, 'the attainment of the highest possible standard and protection."

The regional agency is charged with the responsibility of safeguarding the health of the peoples in the sub-region through initiation and harmonisation of relevant policies of Member States, pooling of resources, and in cooperation with one another, maintaining a collective and strategic focus on important health problems of the sub-region.

WAHO has, through its strategic programmes, undertaken measures to combat malaria, malnutrition, HIV/AIDS as well as maternal and infant mortality. It has also spearheaded the prevention of blindness, increased access to medicines and vaccines, epidemiological surveillance as well as training and health information management in the sub-region.

Through its second strategic plan, WAHO is currently implementing various cutting-edge programmes in the sub-region to improve the overall health systems, ensure high-quality health services, develop sustainable financing of health and support institutional development within WAHO itself.

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Abbreviations

Adopted for source World Health Organization (1, 2)

CBD	Convention on Biological Diversity
CPB	Cartagena Protocol on Biodiversity
DEFRA	Department of Environment, Food and Rural Affairs of the UK
EFSA	European Food Safety Authority
EIR	Entomological inoculation rate
ERA	Environmental Risk Assessment
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FNIH	Foundation for the National Institutes of Health
GDMM	Gene drive-modified mosquito
GMM	Genetically modified mosquito
GMO	Genetically modified organism
HT	Horizontal transfer
IA	Impact assessment
IBC	Institutional biosafety committee
INRSP	Institut National de Recherché en Santé publique
IRS	Indoor residual spraying
IRSS	Institut de Recherché en Sciences de la Santé
IVM	Integrated vector management
LMO	Living modified organism
NASEM	National Academies of Science, Engineering and Medicine
NBA	National Biosafety Authority
AUDA-NEPAD	Africa Union Development Agency-NEPAD
NTO-	Non-target organism
RA	Risk Assessment
RC	Risk Communication
RM	Risk Management
SIT	Sterile insect technique
SOP	Standard operating procedure
WHO	World Health Organization
WHO-TDR	Special Programme for Research and Training in Tropical Diseases of the World Health Organization
WTO	World Trade Organization

Foreword

The Africa Union Development Agency-NEPAD has continued to provide biosafety services to Africa Union Members countries through its programme Africa Biosafety Network of Expertise (ABNE) since 2007. The role of the AUDA-NEPAD biosafety programme is to provide biosafety services to Africa's regulators and policy makers, empowering them to conduct biotechnology research in a safe manner to humans, animals, and the environment and to make informed sovereign decisions in the adoption of biotechnology to foster socio-economic development. Twelve years since its inception, the AUDA-NEPAD biosafety programme focuses on developing a functional Biosafety Network among Africa Union Members countries mainly focused on crops and whose outcomes advance the realization of Africa's Agenda 2063, seven Aspirations and 20 goals.

However, Africa is also burdened with health challenges and globally, Africa shoulders most of the global Malaria burden. Experts are of the view that control and elimination of malaria will need the use of all current control measures with the augmentation of new and emerging technologies such use of gene drives. Given that the current research in the use of gene drives to control malaria is quite advanced, it is timely that the AUDA-NEPAD Biosafety programme leads Africa in the development of the Risk Analysis Guidelines to be used in the development and deployment of the gene drive and other emerging technologies for control and elimination of malaria.

The layout of the Guideline entails Risk Analysis for GMM covering background information current research methods for controlling malaria covering two malaria GMM control strategies- population suppression population replacement. It describes the phased approach, which is a highly recommended framework for the development and deployment of GMM and covers key components of Risk Analysis. It provides a two-fold approach to Risk analysis to produce safety data needed for deployment of the technology and moving from one phase to the next. It also describes risk analysis procedures needed to ensure that the research is conducted in a safe manner for human health, animal health and the environment. To complement the risk analysis, the Guideline outlines how mathematical models could be used to augment risk analysis. It is hoped that the target audience, researcher, regulator, and policy makers in Africa will find this Guideline helpful for research and deployment of the gene drive technology for control and elimination of malaria in Africa.

The Risk Analysis Guideline was developed by a team of competent AUDA-NEPAD staff, referencing key documents of the World Health Organization (1, 2) Guidelines and other professional experts on the subject. However, it should be appreciated that given that there is limited experience in the application of gene drives for the elimination and control of malaria, this Guideline is a living document and will be revised regularly as new scientific information becomes available on the subject.

Glossary

Adopted for source World Health Organization (1, 2)

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 geographical/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 mosquitoes feeding upon a person within some unit of time, such as per day or year.

Fitness – description of the ability to both survive and reproduce, equal to the long-term average contribution to the gene pool by individuals having a particular genotype or phenotype. If differences between alleles of a given gene affect fitness, then the frequencies of the alleles will change over generations, with the alleles with higher fitness becoming more common.

Fixation – a change in the gene pool whereby one variant of a gene becomes established at 100% frequency in the population.

Frequency – an expression of how common a particular gene variant is in the population.

Gene – a segment of DNA that contains information required by cells for the synthesis of a product.

Gene flow – the movement (expressed as an increase in frequency) of genes or alleles into a population from one or more other populations.

Genetically modified mosquitoes (GMMs) (also called genetically engineered mosquitoes, transgenic mosquitoes, or living modified mosquitoes) – mosquitoes that have heritable traits derived through the use of recombinant DNA technology, which alters the strain, line or colony in a manner usually intended to result in a reduction of the transmission of mosquito-borne human diseases – see also Genetically modified organism. GMMs are also likely to be characterized by introduced heritable marker traits to facilitate monitoring upon release into the environment and, in some cases, may include only such markers, as for population biology studies.

Genetically modified organism (GMO) (also called living modified organism) – any organism that has in its genome novel DNA of endogenous, exogenous or mixed origin that was made using modern recombinant DNA technology. Although successive selective breeding of strains of organisms with naturally occurring allelic variations also results in strains with genotypes that differ from the natural population, these are excluded from this definition. Genotype – the genetic constitution of an organism.

GMM system – a transgenic construct incorporated into a mosquito.

Hazard – an event, activity or other cause of a negative consequence or impact identified in a risk analysis.

Horizontal gene transfer (HGT) – heritable transfer of a functional genetic element from one organism to another without mating, most often relating to genetic exchange between different species.

Integrated vector management (IVM) – rational decision-making for optimal use of resources for vector control. The aim is to improve the efficacy, cost-effectiveness, ecological soundness and sustainability of vector control activities against vector-borne diseases.

Introgression – the transfer of genetic material from one organism to another through hybridization.

Non-target organism – any organism that is not a direct target of an intended intervention. For GMMs, the direct target organism is other mosquitoes of the same species in the wild population.

Off-target effects – the outcomes of actions that are not directed to the purpose of the action, whether anticipated or not, possibly affecting either target or non-target organisms. Off-target effects may have negative, neutral or positive impacts on the intended purpose.

Pathogen – an organism that causes disease. In dengue infection, the pathogen is a virus. In malaria infection, the pathogen is a unicellular parasite.

Penetrance – the frequency at which a trait is expressed in individuals carrying a particular gene associated with the trait. Persistence – a descriptor of how long the genetic modification system remains effective.

Population regulation – maintenance of a population around or near an equilibrium level, such as by density-dependent factors.

Population replacement (also called population modification, population alteration or population conversion) – strategies that target vector competence with the intent to reduce the inherent ability of individual mosquitoes to transmit a given pathogen.

Population suppression (also called population reduction) – strategies that target vector density 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.

Risk – an objective measure of the product of the likelihood and consequences of a hazard, defined within a prescribed set of circumstances. Risk is often described as a probability distribution of a set of consequences over a defined time period. Risk analysis – the process of risk identification, risk assessment, risk management and risk communication.

Risk assessment – a methodological approach to define and characterize hazards and to estimate the exposure or likelihood of each hazard occurring, as well as the potential adverse impact of the hazard (harm).

Risk communication – the process through which risk concerns and risk tolerance are articulated by relevant stakeholders and the results of risk assessment and risk management are communicated to decision-makers and the public.

Risk management – the process of identifying and implementing measures that can be expected to reduce risk to an acceptable level.

Self-limiting – GMM approaches in which the genetic modification will not pass on indefinitely through subsequent generations.

Self-sustaining (also called self-propagating or self-perpetuating) – GMM approaches in which the heritable modification is spread and maintained indefinitely through the target population.

Sterile insect technique (SIT) – the inundated release of factory-produced sexually sterile insects into wild native insect populations so that there is a high ratio of sterile males to wild females. Sterilization is usually accomplished using radiation or chemicals. The effect is population suppression, and the effort is most effective when continual and over large areas to reduce the effects of fertile immigrants. Release only of males is preferred, although the release of both sexes has also been effective. SIT has been applied most widely against agricultural pests.

Threshold – the proportion of GMMs, with respect to the total population of the target mosquito species, which will reliably initiate establishment and spread of the modification to high frequency by mating.

Traits – phenotypes that result from single or multiple genes and their interactions with the environment.

Transboundary movement – movement across national, state or other political lines of demarcation.

Transgenic construct – a piece of DNA that has been integrated into the genome of the recipient organisms, typically consisting of a promoter and/or enhancer to provide the desired spatial and temporal pattern of transgene expression, one or more genes to be transcribed, and sequence to stop transcription.

Vector competence – the ability of a vector to become infected with, maintain and transmit a pathogen.

Vector mosquitoes – mosquitoes that are able to transmit a disease-causing pathogen.

Vectorial capacity – a description of the potential for a vector to transmit a pathogen, taking into account vector survival and biting rate, the ratio of mosquitoes to human or animal hosts, and the period of time between when the vector ingests the pathogen and when it becomes infectious for a new host.

Executive Summary

This Risk Analysis Guideline is a guiding document for researchers, regulators and other policy makers involved in GMM research in Africa. It consists of five main categories of topics. First, an introduction to the importance of malaria in Africa and the need to augment all several available tools for its control and elimination. Second, it is an outline of the phased approach to research and deployment of GMM in Africa that is recommended and should be adhered to. Third, it outlines the components of Risk Analysis and its relevance to GMM in Africa. Fourth, it describes how Risk analysis interphases with each phase of GMM product development and deployment steps. Fifth, it outlines the use of mathematical modelling in enhancing the process of Risk analysis.

The process of Risk analysis is partitioned into two sections that are complementary. First, Risk analysis to produce safety data that will be important in decision making prior to deployment of GMM and also used to justify moving from one phase to the next. Seconds, risk analysis to ensure that the experiments are conducted in a safe manner to humans, animals and the environment and avoid adventitious release of GMM prematurely. It is also important to note that during the early phases of the experiment, the developer may not have concrete data on safety but will outline the risks involved and put in appropriate management measures to preclude risk to humans, animals, and the environment to enable them to conduct the study.

It is also notable two strategic approaches to development GMM, and mosquitoes developed through different strategies will differ in their ability to persist in the environment and to spread the inserted genes into a local mosquito population (3). The Risk Assessment requirements and criteria will depend on the specific characteristics of GMM, and the strategy used (3). Two key strategies are currently under research. Self-propagating strategy, also called self-sustaining strategy, rely on gene drive systems that promote the spread and persistence of the transgene throughout the mosquito population of the same species. Self-limiting strategy controls the mosquito population by suppressing their populations or reducing their competence. This Guideline is general in approach and can be used for Risk Analysis of any of the two. However, the strategy for suppression has been cited in problem formulation as a case study to serve as an example in initiative steps in problem formulation (3).

One important key step in developing Risk Analysis data for environmental release of GMM is the use of Problem formulation, first starting with identification of projection goals, identifying potential harm, pathways to harm and later quantification of risk for only Problem formulation is specific to the technology used in GMM strategy use and the construct used in development the GMM hence the need for case-by-case problem formulation and subsequent risk analysis. The Guideline gives an example of Problem formulation recently reported by Connolly et al. (4) based on population suppression gene drives as a pragmatic example, and reports on forty-six potential pathways to harm that will be tested, relevant ones studied further, and it will be revised as more data is obtained. In addition, World Health Organization revised in 2021 (second edition) provides generic outlines of risk to be determined, which are pertinent and good references.

Section 7.4 Outlines risk assessment with terminologies used by the EFSA and Convention on Biodiversity (5 have been outlined. For example, The Convention on Biodiversity (3) publication cites components of risk analysis to include persistence and invasiveness of GM insects, including vertical gene transfer (VGT), horizontal gene transfer (HGT), interactions of GM insects with target organisms, interactions of GM insects with non-target organisms (NTOs) among others. These references have been maintained because depending on exposure, some stakeholders will be looking at risk analysis that reflects these aspects. However, if thorough and plausible problem formulation is conducted and subsequent risk assessment steps are done, the frequently asked safety questions addressing risk concerns such as VGT, NTOs and HGTs should be addressed.

Introduction

Malaria is considered the world's most important parasitic infectious disease. Estimates of malaria-related deaths in 2010 ranged from 655,000 to over 1.2 million (6), with the majority of deaths occurring among African children under five years of age. In 2016, 216,000 million cases were reported with 445,000 deaths, while in 2019, an estimated 229 million cases and 409,000 deaths were reported (7, 8). Children aged under 5 years accounted for 67% (274,000) of all malaria deaths worldwide in 2019. The WHO African Region carries the highest share of the global malaria burden, and in 2019, the region was home to 94% of malaria cases and deaths. In 2019, the total funding for malaria control and elimination was estimated at US$ 3 billion, out of which the government's contribution in endemic countries was US$ 900 million (31%) (8). The International Roll Back Malaria partnership has pledged a goal to "eradicate malaria worldwide by reducing the global incidence to zero through progressive elimination in endemic countries (9). Yet it is acknowledged widely that this goal will not be met without new tools (10, 11, 12, 13). A recent breakthrough in the development of malaria vaccine with an efficacy of 77%, when tested on 450 children exceeding the WHO threshold of 75%, is an added new tool for eradication and elimination of malaria that should be harnessed (14).

Reduction or complete elimination of mosquito vectors is one of the most effective ways to reduce the transmission of disease in endemic areas. Application of mosquito population reduction methods was central to the successful elimination of malaria transmission in Italy and the United States of America in the early 20th century (15) and, transiently, of dengue in the Americas in the early 1960s (16). For more than two decades, scientists have been working to harness the promise of molecular biology to develop genetically modified mosquitoes (GMMs) for use as public health tools to prevent the transmission of these diseases (9, 17, 18, 20).

Recent advances in the development of GMMs have raised hopes for the availability of new potent and cost-effective tools to aid in the fight against malaria and dengue (18, 19, 20). Data on which to base an evaluation of the protective potential of GMM can only be collected through testing, including testing under the natural conditions in which the technology would be utilized. Without the ability to conduct careful and stepwise testing, no new technology can be brought to fruition for the public good. However, given the novelty of GMMs, concerns have been raised about the need for thorough, thoughtful, and transparent preparation for and conducting of field trials (21), and frameworks for environmental risk assessment (ERA) have been produced at various levels (examples are provided in Section 3. Biosafety, and in (22).

Since 2001, scientists involved in this research have, with the support of TDR, the Special Programme for Research and Training in Tropical Diseases (WHO-TDR) and other funders, gathered periodically to consider issues relevant to testing and implementation of genetically modified vectors (1, 2). Through such discussions, a broad agreement has been reached within the scientific community on two tenets, which thus far have been observed.

- First, field-testing should begin with the release of sterile or otherwise self---limiting modified male mosquitoes to gain experience with the technology under circumstances where its effects can be controlled by halting releases (23). Field releases of GMMs carried out to date have focused on the testing of non-replicating, functionally sterile males (which do not bite).
- Second, testing of modified mosquitoes incorporating gene drive should begin under physical confinement (24, 25). No GMMs designed to replicate and spread the modification to wild-type mosquitoes have yet been tested outside of the laboratory.

Site Selection - Experimental and Receiving Environmental

For GMM designated for Africa, the experimental sites are located within the receiving environment. The experimental sites will be at three institutions- i) de l'Institut de Recherché en Sciences de la santé (IRSS) IRSS at Bobo-Dioulasso in Burkina Faso, in Mali; ii) the L'Institut national de recherché en santé publique (INRSP, Target Malaria and iii) The Uganda Virus Research Institute at Entebbe Uganda (26).

Site Characteristics

- To answer risk analysis related questions, it is important to collect baseline data on the site experimentation and potential areas for technology deployment. What constitutes baseline data for Risk analysis could be informed by the problem formulation and especially focusing on parameters with a clear pathway to harm, and this data will support safety characterization. Developers working closely with regulators and risk assessors could work in harmony to ensure what data should be collected and stored (2). To measure the consequence of introduced GMMs will be based on local mosquitoes' biology and ecology (James et al. 2020) and ecological factors that affect the mosquito's population. A broad overview of the type of baseline data has been enumerated (2). It cannot be overemphasized that without good and reliable baseline data on the experimental site and later on the deployment site, it will be impossible to measure risk analysis of the impact of GMMs and therefore impair knowledge-based decision making in technology deployment.

Example of l'Institut de Recherché en Sciences de la santé

- L'Institut de Recherché en Sciences de la santé (IRSS) is located in Ouagadougou in Burkina Faso (27). Burkina Faso is among the top ten countries with the highest malaria cases and death globally (28, 29). Malaria constitutes 43% of the health provider consultations and 22% of the deaths (29). Burkina Faso is among 20 countries where exposure to malaria during pregnancy was >30%, maternal anaemia was >40% in 2018 (29), and approximately 50% of the children under five years have moderate and severe anaemia (29). In the region where IRSS is located, malaria is holoendemic, with transmission peaking during the rainy season with uncomplicated falciparum malaria at around 56%; the entomological inoculation rate was approximately 697 infectious bites per person per year in Bama in 1999. The climate is characterized by a rainy season (May–October) and a dry season (November–April).

Use of Comparators

- To measure the effects of genetic change on the GMM, it is important to compare it with the untransformed wild type. This phenomenon of comparators is borrowed from the development of GM plants and have been wildly published on, from the perspective of crop biotechnology. One of the suggestions is that during the early phases of GMM, the best comparator to use in the untransformed, closely related counterpart is the ancestral laboratory line from which the GMM was derived (1, 2). This will give precision in determining the effect of the genetic change in GMM. However, during field testing, unmodified field derived strains with the same genetic background as GMM will be the most appropriate comparators risk assessment (1, 2).

Phase testing approach of genetically modified mosquitoes

Several Scientific expert groups, including WHO Guidelines for testing GMM and the US National Academies for Science, Engineering and Mathematics Report, proposed the need for phased testing pathway stepwise approach to guide and conduct research from the laboratory to deployment (1, 24, 25, 30, 31, 32, 33, 34). This entails beginning with studies in containment facilities, followed by field cages or confined field studies to answer further research questions that will support open field release (30). Following several consultative meetings, the concept of phased testing of GMM was recommended and developed, as illustrated in figure 1 (1, 2). GMMI testing in Integrated Vector Management (IVM) in Africa should adhere to this phased testing approach outlined in the following section. Four phases of testing GMMI have been proposed, and this includes:

- Phase I
- Phase II
- Phase III
- Phase IV

The main purpose of phased testing is to allow the research to gather satisfactory safety and technology efficacies data on GMM technology before moving to the next phase. The following is an outline of the different phases in GMM research and deployment. It is important that the research comprehends the proceeding four phases of research and deployment because the risk analysis is closely related and intertwined to the four phases. The following section gives details of the four phases.

Phase I: Laboratory Testing under containment

Phase I will comprise small-scale laboratory testing to determine efficacy and safety. This will be followed by testing a large population in larger cages in a laboratory setting conducted under appropriate containment facilities and procedures as determined by containment Guidelines. The testing step aims to demonstrate that the GMM has desired biological and functional characteristics with respect to efficacy and safety (1, 2).

Phase II: Containment (Greenhouse or screen house) or confinement (Ecological)

Selected and promising GMM from phase I will proceed to phase II testing. Phase II testing will comprise confined testing in a more natural setting that limits release into the environment. In phase II testing, one option will be to conduct small scale testing under physical confinement (containment) within a large cage that mimics the disease-endemic conditions while precluding the possibility of GMM escape into the environment. Physical containments could be achieved using greenhouse or screen houses or where conditions allow, small ecologically confined sites could be used. The decision on these sites will be made by the Burkina Faso, Mali, and Uganda Governmental National

Regulatory Authorities. Ecological isolation as an option will solely be feasible in Uganda, which has several islands within lakes. Given that Burkina Faso and Mali are landlocked, confined and containment use of screen house or greenhouse will be likely the only option (1). Similar to Phase I, the Phase II trial will continue to assess the biological and functional activity of the GMMs, but also include the effect on local/wild type mosquitoes. Data obtained from Phase I testing will contribute to the impact of GMM on malaria control and elimination but is not sufficient enough. This is because the data experiments will be conducted under a limited scale and need to move to phase II testing. However, as testing moves to large scale GMM trials in the environmental and disease-endemic countries, there will be a need to take into consideration and apply the relevant ethical and regulatory practices as outlined in section 4 Ethics and public endearment, and Section 5 Regulatory Frameworks) (1, 2).

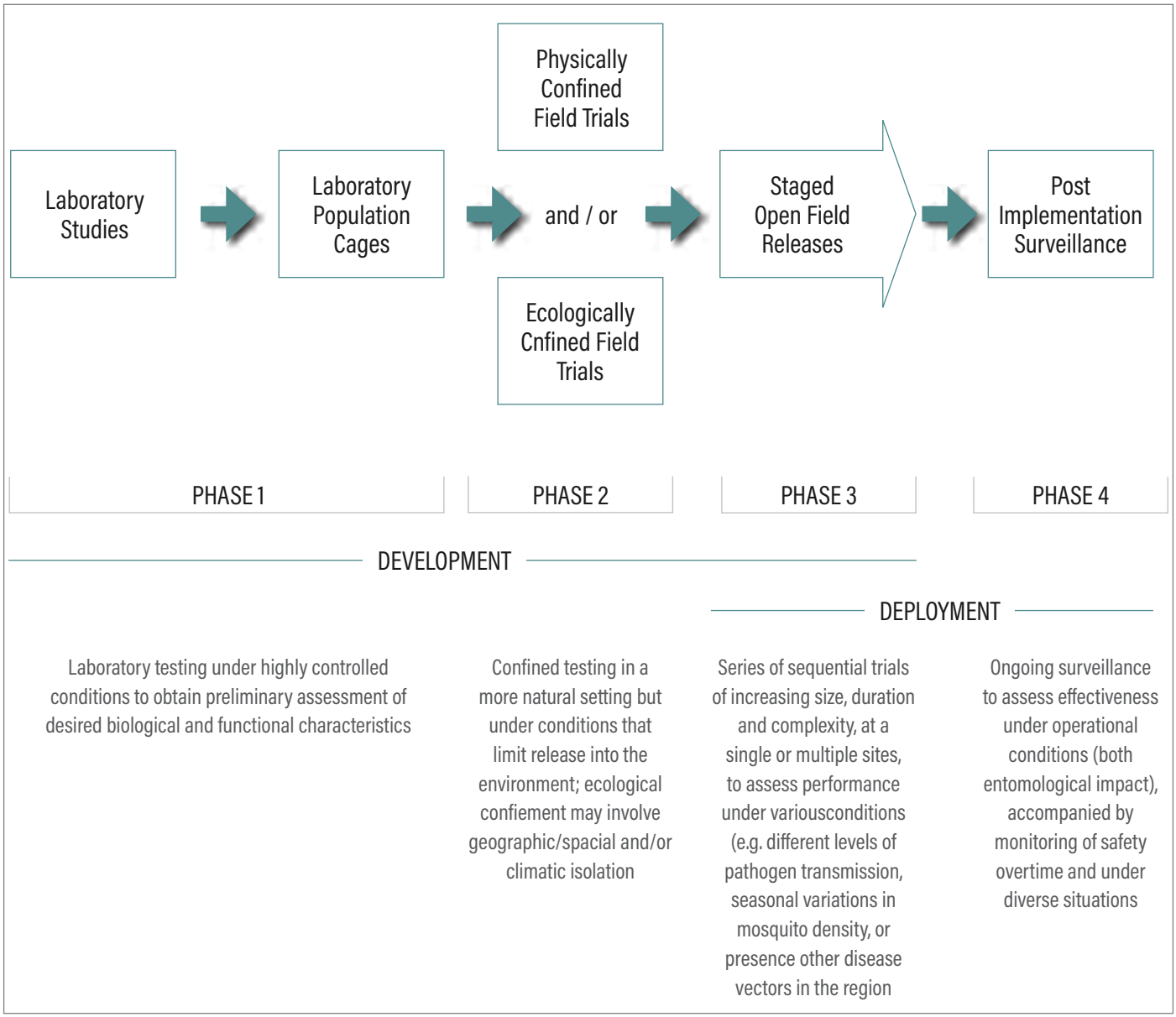


Figure 1: Phased Pathway for Genetically Modified Mosquitoes, Karter SR, and Friedman RM (2016)-35 modification of WHO (2016, 20211, 2).

Phase III: Open Release Field Trials

The GMMI technology may be tested in Phase III upon satisfaction of phase II. This will involve a series of sequential trials of increasing size, duration, and complexity to be conducted at a single site or multiple sites. The sites will assess performance under various conditions, for example, under different levels of pathogens, season variation in population density and presence of other diseases vectors. Parameters to measure will include- entomological parameters, the impact of GMMs on infection, disease in human populations and function and efficacy of GMMs. Phase III will constitute limited deployment of technology, especially in the self-sustaining case. Approval for moving forward in stages 1-3 will be under the National Regulatory Authorities (1, 2).

Phase III will begin with a limited release intended to understand the following parameters-the delivery requirements and the functionality of GMMs. These parameters will be determined under different circumstances, such as-different ecologies and different seasons. This preceding information should be a prerequisite to the large release in Phase IV and will be used in determining- the trial design and interpretation of results obtained in phase IV.

Phase IV: Deployment of GMMs as Public Health Tool

This will be a "wide-scale application" as part of national or regional programmes for vector and disease control. The decision at this phase will involve National Regulatory authorities and may involve additional authorities determining national or regional disease control (1). This part of the GMM study is similar to other public health studies in that GMM will require ongoing monitoring to determine whether their efficacy has diminished with time or because of unexpected effects used in new areas. Appropriate entomological outcomes that guided the GMM deployment must continue after the release. Depending on the type of GMM technology and deployment strategy, multiple year follow up may be required.

GMMs that reach phase IV will go through extensive efficacy testing. Their behaviour in the natural setting will be established by activities in phase III. But it cannot be assumed that they will behave as expected. The analogy to experience with the long-lasting treated bed net, efficacy may change due to several factors such as a change in genetic constitution or external factors such as human activities or weather. In case of reduced efficacy, any second-generation GMMs may be recreated from phase I to III before being monitored in Phase IV.

The development steps have part of the World Health Organization (1) and US National Academies for Science Engineering and Mathematics Report of 2016 outlined in Figure 1 (1, 34).

As mentioned in the preceding section, the Risk analysis is outlined based on the phases of the framework. However, considerations of specific risk corresponding to each testing phase should be outlined so that risk analysis is commensurate with the regulatory requirements applicable to each phase. This will match risk analysis to each corresponding phase and therefore limit taking into account unnecessary considerations risks that are not relevant to particular phases.

Components of Risk Analysis

The components of risk analysis have been described under several venues such as the USA Environmental Protection Agency (36), The Convention on Biological Diversity (CBD) (3), Cartagena Protocol on Biosafety (37), the European Food Safety Authority (38), Australian Office of Gene Technology Regulator (39), United Kingdom's Department of Environment, Food and Rural Affairs (DEFRA). Risk analysis in an integrated process is made of three main components (i) Risk assessment (RA); (ii) Risk management (RM); (iii) Risk communication. The interrelation between these components ensures a well-functioning of the Risk analysis process, which in turn forms the basis for decision making. GMO and organisms such as GMM may raise many potential concerns as for their safety to people and the environment, public acceptance, or ethical considerations. Risk analysis is a case-by-case process. Particularly, in the frame of phase testing approach of GMM, risk analysis will be less complex in the laboratory testing and small-scale confined phases but will be more complex in open field release phases. Care should be taken by the regulator to clearly focus and identify appropriate potential risks relevant to each specific phase.

Risk assessment

Risk assessment (RA) is at the core of risk analysis and has been explicitly described in several documents (3, 5), among others. The key guiding principles of risk assessment include- risk assessment should be conducted in a sound scientific and transparent manner; risk of LMO should be considered in comparison with the risk posed by non-modified recipients of parental organisms in the likely receiving environment and should be conducted on a case-by-case basis (3). It is a case-by-case process that primarily deals with risk concerns related to the new characteristics of the technology. Environmental risk assessment of GMOs usually follows a multi-step process, and a stepwise approach has been recommended depending on the authorities. 5 to 6 steps are elucidated that include (3, 5, 40).

1. Problem formulation, including identification of hazard and exposure pathways,
2. Hazard characterization,
3. Exposure characterization,
4. Risk characterization,
5. Risk management strategies,
6. Overall risk evaluation and conclusions

Problem Formulation

Environmental risk assessment of gene drives should begin with a thorough problem formulation. Several authors and reputed sources have identified key steps to problem formulation (3, 5, 36, 41, 42, 43, 44). These include-

1. Identifying protection goals, assessment points and measurable points; the latter is also referred to as harm.
2. Identify unique genotypic or phenotypic characteristics in GMM that are not found in the comparator that could cause harm.
3. Determine if there are pathway (s) on how the release of GMM could cause the harm(s) identified,
4. Formulating risk hypotheses about the likelihood and severity of the effects of the harm(s),
5. Obtain existing information that could test the hypothesis to determine if the GMM could cause harm.
6. If information from 5 is insufficient, develop experiments to test the hypothesis for decision making.

Problem Formulation and Research and Deployment site selection

Problem formulation takes into consideration the research sites and deployment areas of the GMM. Research in GMM in Africa is being conducted in three countries, Mali, Burkina Faso, and Uganda, while the environmental release of GMM will be made in seven countries that enacted Biosafety Acts. Protection goals should be obtained from the National Environmental Policies of these countries, and problem formulation should involve stakeholders from these seven countries of GMM deployment.

Approaches to developing Genetically Modified Mosquitoes

Two main approaches to development of GMM for the control and elimination of malaria include population suppression, which involves strategies to reduce or eliminate an insect population (45). Population Replacement, also called modification of alteration, replaces existing wild mosquito populations with strains or species that are innocuous in terms of pathogen transmission (45, 46).

Population Replacement

First is a self-propagating strategy, also called self-sustaining strategy, which relies on gene drive systems that promote the spread and persistence of the transgene mosquito population of the same species (1, 46). This involves the introduction of engineered DNA and/or the manipulation of endogenous mosquito genes in a way that would inhibit parasite or virus replication and thus reduce vector competence. When released into the environment, these GMMs would introduce the change into the local mosquito population through mating and ability to integrate the inherent trait into the local population resulting in pathogen reduction or elimination through inability to survive in the host (1). Replacement strategies are intended to be heritable and spread through the target population and persist in the ecosystem at least in the medium term (3).

Population Suppression

The second is the self-limiting strategy that controls the mosquito population by suppressing their populations or reducing their competence (2, 3). These approaches include methods to reduce the number of female mosquitoes (with or without a concomitant direct effect on males), which could result in decreased reproduction and a decline in the population (1) or interrupting larval development into offspring (3). This could be accomplished through biasing against the development of female progeny (sex-ratio distortion), reducing female fertility, or shortening the lifespan of female mosquitoes, thereby decreasing the length of time available to reproduce and transmit a pathogen from one person to the next (1). Self-propagating strategies rely on gene drive systems that promote the spread and persistence through the population of the same species (3).

Paratransgenesis- population Suppression or Replacement

Paratransgenesis can be targeted to either population suppression or replacement (CBD, 2016). The approach genetically transforms insect-associated symbiotic microorganisms, a process called the paratransgenic method (47). Paratransgenesis can target transgenesis of symbiotic bacteria, fungi, or viruses of the vector insect and result in genetic manipulation that delivers effector proteins that block development or transmission of the pathogen (48, 49). The ultimate goal of paratransgenesis is to combat the disease vectored by the insect, thereby reducing its ability to damage human health or economic interests (50, 51, 52, 53, 54). Paratransgenesis strategy could reduce,

eliminate, or control the capacity of vectors to transmit the pathogen by blocking the development of the pathogen in the vector. This Guideline is general in its approach and can be used for Risk Analysis of any of the three strategies.

Protection Goals and Target Countries

Protection goals for African Union Member countries are published for each specific country in respective National Environmental Policy Documents. For countries where research is being conducted, namely Burkina Faso, Mali and Uganda, protection goals broadly include biodiversity conservation ecological functions (55, 56, 57). Receiving countries of GMM are outlined in section 2.4 of this Guidance Document. This Guideline gives an example of Protection Goals for Burkina Faso (57).

Example of Protection Goals in Burkina Faso.

Burkina Faso Ecosystems consist of agricultural ecosystems, pastoral ecosystems, wetlands, urban areas, mountain ecosystems and conservation areas. These ecosystems host large biodiversity, including 128 species of mammals, 516 species of birds, sixty species of reptiles and amphibians, 121 fish species, 1,515 species of insects and 1,951 species of flora. Burkina Faso's threatened species include panthers, elephants, crocodiles, and pythons. Three main threats to biodiversity in Burkina Faso are high deforestation (4% forest cover), reduction in freshwater bodies and wetlands and decreased yields from agricultural systems (57).

Example of Problem Formulation using Population Suppression strategy

Preliminary problem formulation for gene drive mosquitoes for control of malaria in Africa has been conducted by several teams (58, 59, 60) for the release of GMM gene drive modified D. suzukii carrying a suppression drive. Four categories of identified protection goals for target areas were: biodiversity conservation, protection of human health, protection of animal health, water quality and one ecological aspect (59). Recently Connolly et al. (4) published a plausible analysis of forty-six pathways to 'Harm' covered under section 2.4 that will be used as an example of a case-by-case approach to problem formulation.

Plausible Pathways to Harm for release of population Suppression Gene Drives

Initial steps in environmental risk assessment (ERA), performed for simulated field releases of the dsxFCRISPRh transgene in West Africa for the control of the human malaria vector Anopheles gambiae in West Africa, have been developed (2). A total of forty-six potential pathways to harm resulting from deployment of gene drive mosquitoes with population suppression gene drives containing CRISPR-Cas9-based transgene homing at the doublesex locus (dsxFCRISPRh) have been proposed by Target Malaria. Two pathways have the potential to cause harm to water quality, eight pathways to biodiversity, sixteen to animal health and twenty to human health. The authors also report on 'risk hypotheses' for critical steps in each pathway and an 'analysis plan' for setting out evidence that could be used to test each risk hypothesis. The developed pathways will inform the next stages of an ERA on population suppression gene drive, which will involve assessments of the likelihood and magnitude of the identified potential harms (4).

Hazard Characterization

Hazard characterization is defined as the qualitative and/or quantitative evaluation of adverse effects of the harm on the environment or human health, or animal health (5). Hazard characterization should include only 'Harms' with a clear pathway to adverse effects on the environment human and animal health. The researcher/applicant will determine a quantitative or qualitatively evaluation of the 'Harm' identified under the problems formulation step above. For example, for expressed proteins, this will involve conducting toxicological and allergenicity studies using the standard international prescribed protocols. Table 3.1 and 3.2 gives examples of the potential types of harm that could be assessed in quantitative or qualitative terms (1, 2). However, it is noteworthy this assessment has to wait until the final problem formulation is conducted to determine 'Harms.' The magnitude of each adverse effect should, where possible, be expressed in qualitative terms (61, 62). An ordered categorization could also be used such as "high", "moderate", "low", or "negligible", where the ordering is from 'high' at one end to 'negligible' at the other (5, 63). However, notes should be provided to facilitate the translation of this 'ordered categorization' into quantitative terms.

Exposure Characterization

The process of problem formulation identifies direct and indirect (direct or indirect exposure) routes through which the harm may be imparted to the environment, human or animal health. Exposure characterization involves an estimation of the likelihood of the occurrence of the adverse effect (5). Exposure characterization involves the determination of the nature, magnitude, frequency, and duration of the exposure to the GM animal by the applicant (5). Qualitative expression using ordered categorical description such as high, moderate, low, and negligible may be used or quantitatively relative measure of probability from 0 to 1 where 0 is an impossibility and 1 certainty (5).

Risk Characterization

The risk assessor should characterize risk by combining the Exposure categorization of (Negligible, Low, Moderate, High) with the Hazard characterization categories (Negligible, Low, Moderate, High) as should below table 3.

Table 1: An Example of Risk Characterization (Hazard x Exposure)

Hazard	Exposure			
	Negligible	**Low**	**Moderate**	**High**
High	1	4	6	7
Moderate	1	3	5	6
Low	1	2	3	4
Negligible	1	1	1	1

It is recommended that risk uncertainties are taken into consideration to cover but not limited to assumptions, any conflicts in scientific literature or viewpoints (5). Furthermore, the risk characterization should indicate whether the problem formulation, hazard characterization and exposure characterization are complete, in which case no further analysis is necessary (5).

Risk Management

After assessment of Risk and taking into consideration, the areas of uncertainty, risks management measures should be proposed. The risk management strategies aim to reduce the identified risks associated with the GMMI to a level falling within the limits of concern related to the particular receiving environments and should consider the areas of uncertainty identified during the ERA. Risk management focuses on selecting and implementing plans or actions to ensure that risks are appropriately managed. The risk management plan is established and reviewed, and when necessary, additional management measures may be added to the risk management plan. Preventive management measures are prioritized over measures directed to harm reduction or repairing (5, 39).

Risk Management Strategies

Table 2: Main steps of risk assessment

Step	Key points to consider
1. Risk identification	• What could go wrong? • What harm to the protection goals could be expected?
2. Risk characterization	
2.1. Likelihood assessment	What is the overall likelihood of the risk based on the likelihood of individual steps of the risk scenario?
2.2. Consequence assessment	How serious is the harm as assessed through particular risk scenarios
3. Risk evaluation/conclusion	• What is the level of concern? Is the risk negligible? Low? Moderate? High? • What control measures are required?

Risk Communication

Risk communication is the exchange of information, ideas and views between the regulator and stakeholders. It is a continual and interactive process to provide, share or obtain information and to engage in dialogue with stakeholders regarding the analysis of risk within the context of the legislation. Therefore, it conveys the rationale for decisions made by the regulator. Because risk communication is a dialogue between the regulator and the stakeholders, it is important to distinguish it from public engagement done by the technology project team, even if the target people may be the same. The overall goal of risk communication is to promote trust and credibility in the ability of the regulator to effectively regulate the technology.

Risk communication is a complex process, yet it is a key part of risk analysis. It is a two-way process in which the regulator recognizes the wide range of stakeholders' views, not attempting to change basic values and beliefs. Despite the sophisticated technical language of science and technology, the regulator's message should be appropriate and avoid jargons as much as possible. The Regulator recognizes and accepts that the community may have a wide range of views on the technology. All issues and concerns within the scope of technology legislation should be considered.

Risk communication usually focuses on potential risks from technologies and how to manage them so that the undesired consequences do not occur or are minimized. In the context of GMMs, it is important that risk communication takes into account the foreseen benefits of the technology, i.e., efficient malaria control. This aspect may not be part of the risk analysis per se, but it is a key condition for the acceptance and adoption of the technology.

Key Elements of Risk Analysis at different Testing Phases

Unavoidably, various potential hazards will be identified. The phase testing approach will allow the partitioning of these hazards in accordance with the specific phase. Risk analysis in earlier phases will benefit the later phases in updating the risk conclusions. The regulator's attention should be fully drawn to potential risks pertaining to each specific phase so that no major risk remains unassessed.

Infra2Horizon (64) identified six areas of concern in assessing the risk of any activity when working with infected mosquitoes or other arthropods-

1. Risk of escape of mosquitoes into the environment
2. Risk of the mosquitoes biting laboratory workers.
3. Risk of pathogen transmission between individuals, for example, laboratory workers to community
4. Risk to public health, animal health and environment of the biological agents in the mosquito infecting the laboratory workers and the environment.
5. The availability of treatments and preventive measures.

In developing the application to conduct GMMI study for approval by National Biosafety Authorities (NBA), the applicant will do a thorough risk assessment mostly based on published literature to determine the levels of risks posed by the above 1 to 5 and propose risk management measures and communication approaches to ensure that the identified risks posed 1 to 5 are mitigated. It should be noted that in the early phases of GMM research, the applicant may not have a clear picture of the magnitude of risks posed. However, provided the NBA is satisfied with the proposed management and communication measures put in place, the application should be approved with terms and conditions.

Phase I Laboratory studies including laboratory population cages

During Phase I, the GMMI studies will be conducted under physical containment, which will comprise either a laboratory or an insectary. Specific containment conditions suitable for the experimentation are outlined in the IVM Guideline for Containment Facilities for Testing GDAs.

The containment level will be determined when the applicant makes the application and conducts risk assessment in which and proposes the management measures. This is because at Phase I stage, there is limited information on the stability of the GMMI, limited information on the genetic modification and uncertainty on the hazards (1, 2).

The RA will establish conditions under which the study should be conducted to ensure an acceptable level of exposure of the personnel, security to stop GMMs from escaping either through flying or early developmental stages such as egg, larva or pupa and use of appropriate methods of disposing of the waste (1). Specifically, the RA and RM will be focused on the following-

- Ensuring adequate containment procedures: insect-proof cages and design and equipment of the insectarium
- Regular and periodic training of research personnel and other staff working in premises in RM conditions.
- Arrangement for appropriate disposal of waste
- Ensuring that all involved in the GMMI study are in good health.
- Ensuring that staff wear appropriate clothing as prescribed in Containment Guidelines.
- Ensuring that appropriate standard operating procedures (SOPs) are documented and observed.
- Ensuring reduced exposure of personnel and animals to biting by mosquitoes.
- Putting in adequate monitoring procedures to ensure that RA and MA conditions are enforced.
- Putting in appropriate contingency measure to respond to unexpected escape of GMM

Other Study Parameters for Phase I and II relevant to Risk Assessment

It should be noted that during Phase I and II, there are other Risk Assessment studies being conducted in the containment facility. It is this data that is also examined by the regulator to allow the experiment to move from one phase to the next. These parameters under consideration are based on the results of the problem formulation and identified pathways and so cannot be determined at this stage until the final problem formulation is completed; however, WHO (1) Guidelines provide a generalized set of parameters that should be analysed in Phase I and II. In the WHO document, they are referred to as entomological and parameters but based on identification of 'Hazard Characterization' step of risk assessment. The purpose of Conolly et al. (4)'s identification of forty-six plausible pathways to harm is to refine the contents of the WHO (1, 2) so that only relevant hazards with a clear pathway to harm are considered in risk assessment.

Because of high cost of study, the parameters obtained from Problem formulation will be prioritized and selected to focus only on those with clear pathway to harm to be studies further to generate RA data needed to make decisions.

The RA should focus on the hazards (changes that may lead to harm as a result of the genetic modification), the (experimental) methods to measure this and the exposure assessment. References to 'differences' mean differences between the transgenic strain being tested and the appropriate comparator (1, 2).

Table 3: Example parameters that may be relevant in laboratory studies (phases 1 and 2) as part of the RA for transgenic mosquitoes (Source WHO, 1, 2)

Parameters	Example hazards	Assessment methods	Assessment endpoints
Female fecundity	Increased vector abundance	Cohort experiment; life Table analysis	Is it limited by population Density and/or individual physiology? Is there a significant Oviposition rate difference?
Oviposition rate			
Egg development rate	Increased growth potential; educed Predation	Cohort experiment; life Table analysis	Is there a significant difference?
Larval survival			
Pupal survival			
Adult emergence	Increased vector abundance	Cohort experiment; life Table analysis	Does the timing of adult emergence differ significantly?
Adult Size	Increased vector fitness	Increased vector fitness	Is adult size significantly different?
Adult Survival	Increased vector activity; more effective mating potential; increased biting efficiency for females	Cohort experiment. Life table analysis. Population-level modelling	Is it density-dependent? Is it significantly enhanced/ diminished by the modification?
Mating strategy	Increased vector abundance. separation of GM and wild types	Cohort experiment	Is there assortative mating? Are there costs to males/females' gametes? Does the modification affect mating competitiveness?

Parameters	Example hazards	Assessment methods	Assessment endpoints
Sex ratio	Increased female abundance. increased biting potential if more females	Increased female abundance; increased biting potential if more females	Is the sex ratio substantial different from the null Expectation
Flight ability	Increased vector activity; more effective mating potential; increased biting efficiency for females	Cohort experiment. Physiological experiment	Is flight duration or distance significantly different?
Biting rate	Increased disease transmission	Cohort experiment. Physiological experiment	Does the feeding rate
Vector capacity	Increased disease transmission	Cohort experiment. Physiological experiment	Is the capacity to harbour pathogens? Significantly enhanced/diminished?
Insecticide resistance	Increased vector abundance	Standard Insecticide dose Response test	Is it expected to alter the competitive status of transgenic lines significantly? Does it make transgenic lines significantly less amenable to conventional control?

Phase II- Physically and ecologically confined field trials

Promising GMMI from Phase I will move to Phase II testing that will constitute confined testing in more natural testing that will limit release into the environment. In moving from Phase I to Phase II, the applicant will make an application using appropriate forms obtained from the National Regulatory Authority. The application will, among other information, require the provision of data on RA and RM measures. The application, if successful, will be approved with terms and RM conditions to be adhered to while conducting the GMM.

Small testing on Phase II may involve physical confinement (containment) within a large cage to mimic the disease-endemic setting but minimize the possibility of escape. This could be done in greenhouses or screen houses or may involve testing in small ecologically confined sites. The decision on these sites will be made by the Burkina Faso, Mali, and Uganda National Regulatory Authorities after evaluation of the application submitted by the applicants that will include information on risk assessment and management measures. Approval will be given with terms and conditions under which the study should be conducted. The greenhouse or screen house will constitute some of the RM containment measures proposed by the applicants.

After the applicant has done RA, they will propose appropriate confinement measures to ensure safety to the environment, human health, and animal health. Depending on the level of confinement in a greenhouse or screenhouse, the specific conditions to adhere to will be as prescribed in the IVM Guideline for Containment Facilities for Testing GDAs.

Measures should be put in place to ensure no escape into environment, given that the chances of escape are higher in Phase II than in Phase I

The risk management measures proposed in Phase II will be similar to Phase I with key additions including- site security and engagement with people in the site vicinity for proper risk communication

The following appropriate containment measure should be adhered to

- Ensuring adequate containment procedures: Appropriate cage designs and design equipment of the insectarium
- Regulator and periodic training of research personnel in RM conditions with experience on how to conduct the experiment.
- Arrangement for appropriate disposal of waste
- Ensure that all involved in the GMMI study are healthy.
- Appropriate clothing that staff wear appropriate clothing as prescribed in Containment Guidelines.
- Ensure that appropriate standard operating procedures (SOPs) are documented and observed.
- Ensure reduced exposure of personnel and animals to biting by mosquitoes.
- Put in adequate monitoring procedures to ensure that RA and MA conditions are enforced.
- Should put is appropriate contingency measure to respond to unexpected escape of GMM

Phase III- Staged open field releases.

Phase III is a transit stage between product development and product deployment. It, therefore, involves a series of open trials in the environment, which should be designed with increasing size, duration, and complexity (Figure 3, WHO (1). Phase III will enable the technology to be evaluated to determine technology efficacy and more comprehensive RA to determine the safety of the technology. This includes but is not limited to:

- Evaluating the technology under different climatic conditions: For example, mosquito population density is highest in rain season more than the dry season because the former provides water needed for breeding conditions of Anopheles gambiae complex where females need water to lay eggs.
- Evaluation of the technology under high malaria prevalence in rain season compared to the dry season in target countries.
- Evaluation of the technology under different ecological conditions and within one ecological season - you expect high-density vegetation in rainy seasons than in the dry season.
- The technology will also be evaluated under variable fauna - for example, the predators of mosquitoes such as birds,
- The technology will be tested whether there are natural species of Anopheles gambiae complex,
- The technology will be tested under integrated environmental conditions where other methods of malaria control are practiced, such as the use of nets.
- Because of environmental variability, there will be a need to use appropriate experiment designs and data collection measures both in testing technology efficacy and collecting RA data.

Risk Assessment data that could be collected under Phase III

Table 3 from WHO (1) indicates the generic type of RA data that could be collected. Phase III is very critical because the GMMI are released into the open field, though this may be done in a staged process. Several interest groups, including the National Biosafety focal points country of experimentation and deployment, the public especially in the vicinity of the open release, will have an interest in understanding the biosafety status of the technology. Safety data should be available to justify this movement, and various authorities (3, 5) have identified needed categories of safety data under the following categories:

- Persistence and invasiveness of GM insects, including Vertical Gene Transfer (VGT)
- Horizontal gene transfer (HGT)
- Interactions of GM insects with target organisms
- Interactions of GM insects with non-target organisms (NTOs)
- Environmental impacts of the specific techniques used for the management of GM insects.
- Impacts of GM insects on human and animal health.

It is important to note that the above safety concerns are not stand-alone and will be on a case-by-case basis depending on the type of construct used in developing GMI. An example of one construct under research is analysed under Conolly et al. (4). Plausible pathways to harm. Under this analysis, "Hazards" with a clear pathway to harm on human health, animal health, water quality, and ecological factors will be identified, and their likelihood determined. The products of Hazard and respective probability (Risk= Hazard x Likelihood) will provide information on the magnitude of risk for each hazard. The magnitude of

"Risk" post by "Hazard" in conjunction with available Risk Management measures will be key factors Regulators will consider in decision making in the deployment of GMMI in West Africa.

Each of these categories of RA should be addressed through problem formulation, hazard characterization, exposure characterization, risk characterization and proposed risk management measures. It is also notable that through phase I and phase II, part of this data should have been collected and should be enhanced in Phase III.

Interestingly, these steps will benefit from the management of most technical risk concerns (e.g., stability of the technology, the potential impact on non-target organisms, efficacy of the technology, means to stop the dispersal of GMMs when required, etc.) at earlier phases.

However, specific data relevant to the deployment of GMMI in West Africa should be derived from the Problem formulation analysis that will identify the specific hazards developed, and Conolly et al. (4) developed excellent and extensive plausible pathways to harm outlined in the following section relevant to RA of GMMI tested and released in West Africa.

Table 4: Examples of Parameters that may be relevant to open field studies as part of Risk Assessment of GMM- Source: World Health Organization (2).

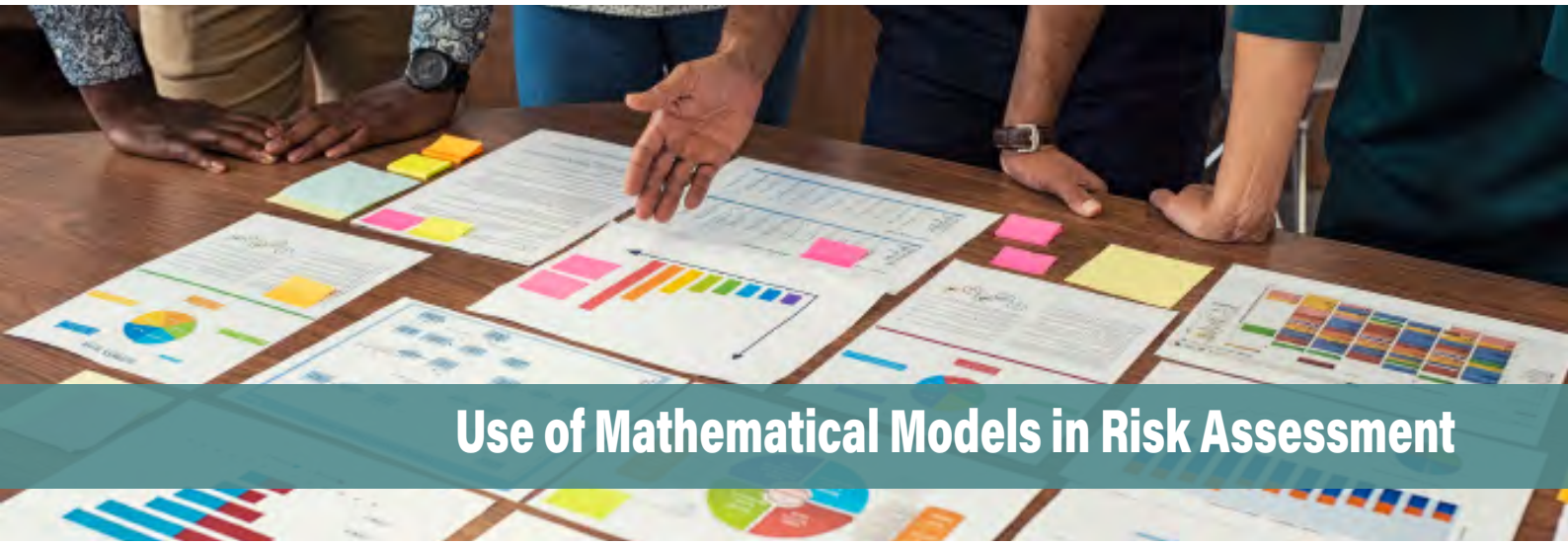
Parameters	Example of Hazard	Assessment Methods	Assessment endpoints
Population size	Increased vector abundance. Ecosystem disruption	Field population monitoring. Population-level modelling	What is the impact of the release? Relationship between release rate, timing, method, and outcome?
Density dependence	Increased vector abundance. Ecosystem disruption	Comparator studies at a range of densities in the laboratory. Field population monitoring; Population-level modelling	Does the transgenic strain differ significantly in the role of this ecological process?
Spatial distribution	Increased vector abundance. Ecosystem disruption	Field population monitoring; population-level modelling; life table experiments	Limits to the spread of the transgenic organism? Rate of spread of the transgenic insect under a range of conditions?
Vector capacity;	Increased transmission per bite	Increased biting rate Comparator studies; post-release monitoring	Is the capacity to harbour and transmit pathogens increased?
Behavioural resistance	Change in behaviour that avoids or reduces the efficacy of conventional management.	Comparator studies; cohort studies on behavioural changes in different life stages; post-release surveillance; population-level modelling	Under field conditions, what limits the appearance and spread of resistance due to mosquito behaviours? Is there potential for assortative mating in the field?

Parameters	Example of Hazard	Assessment Methods	Assessment endpoints
Biochemical resistance	Change in physiology that avoids or reduces the efficacy of conventional management.	Comparator studies; cohort studies on physiological changes in different life stages; post-release surveillance; population-level modelling	Is the likelihood or rate of resistance development enhanced in transgenic mosquito strains?
Mass rearing quality indices	The quality of released insects is different from planned, affecting negative outcomes.	Cohort experiments; comparator studies before release; operational design and audit; pre-release monitoring; post-release monitoring	Do specific aspects of released mosquito quality affect mosquito densities, pathogen transmission and transgene stability?

RA should build on evidence regarding the potential hazards indicated during Phase 1 and Phase 2 trials, the methods to measure those hazards and exposure assessments. Comparator studies aim to compare the GMM with a conventional (unmodified) counterpart.

Post-implementation

After a successful release of GMM into the environment, continuous monitoring of the entire system will be necessary since many unforeseen and undesired consequences may arise. The long-term efficacy of the technology and behaviour of mosquito populations may change over time due to insect evolution. In both insect population suppression and replacement strategies, the genetic modification in gene drive GMMs is likely to persist in the environment for some period. Therefore, resistant individuals may emerge and pose a serious threat in the context of the already known insecticide resistance. The regulator should focus on monitoring if the potential risks identified in earlier phases still remain under control and new post-release risks are adequately managed.



Use of Mathematical Models in Risk Assessment

The objective of modelling in the Risk Assessment context is to predict behaviour based on the properties and assumptions of the transgenic modification and use the predictions to assess the likelihood of events (2). For example, data collected in Phases I and II could be used in modelling to predict the possibility of risk of adverse events related to the spread and dispersal of GMM (2). The prediction could also cover important parameters to answer important questions such as: could the genetic medication result in enhanced fitness of GMM, or could it also model interspecific interaction to predict the ecological impact of GMM when released (2).

Knipling (65) first conceptualized the use of the sterile insect release method (SIRM) for pest population suppression. Knipling (66) theoretical devised simple mathematical models to demonstrate SIRM can result in the total eradication of a defined population few generations. Empirical evidence to support this model has been demonstrated in the eradication of the screwworm (Cochliomyia hominivorax) from the Caribbean Island of Curaçao and peninsular Florida (67) elimination of isolated infestations. It has further been demonstrated in regional suppression against the cotton boll weevil, Antonomus grandis, mosquitoes and the codling moth, Cydia pomonella (68).

Modelling for driving-Y chromosome that damages X-chromosome

This approach uses synthetic sex distortion for controlling mosquitoes causing human malaria. The Y chromosome in the modified male mosquito shreds the X chromosomes in the germline, resulting in gametes predominantly having Y chromosome and a distorted sex ratio in viable offspring with >95% male offspring (25, 69, 70). This approach was reported in A. gambiae (25, 70, 71). Deredec et al. (72), through modelling, have suggested various requirements for the optimal spread

of the gene drive in the population and has also been modelled to determine the requirements for the spread in a population. North (73) provides data on the spatial spread of homing endonuclease gene in the mosquito population.

Modelling for Population Replacement Gene Drives

The third approach is for GMMI is population replacement that introduces a construct increases toward fixation without collapsing the local vector population (25, 69). This introduced construct could conceivably knock out a gene required for mosquito infection by the parasite or knock a gene that provided defence against parasite infection of the mosquito or onward transmission to humans or both.

Smith (74) demonstrated that expression of an anti-Plasmodium effector gene gives transgenic mosquitoes a fitness advantage when fed malaria-infected blood and fitted best-fit models up to twelve generations. Similarly, Mauro et al. (75), under cage experimentation, demonstrated that transgenic mosquitoes resistant to malaria have a fitness advantage over non-transgenic mosquitoes when feeding on Plasmodium-infected blood developed best-fit population genetics models.

Dhole (76) used population genetics modelling to compare the expected characteristics of three spatially self-limiting gene drive systems: one-locus underdominance, two-locus underdominance and daisy-chain drives. Results showed that the daisy-chain system was the most efficient, requiring the smallest release, followed by the two-locus underdominance system and then the one-locus underdominance system.

North et al. (77) used a stochastic simulation model to explore the potential of using a driving-Y chromosome to suppress vector populations in a 106 km2 area of West Africa, including all of Burkina Faso. They explored elimination of the target species in some regions and suppression in others as affected by environmental conditions (with spatial heterogeneity especially in seasonality), mosquito behaviour, and the properties of the gene drive. Results showed that despite spatial heterogeneity, repeated introductions of modified mosquitoes over a few years into a small fraction of human settlements could de adequate substantially reduce the overall number of mosquitoes across the entire geographic area.

Modelling in the Risk Assessment context is to predict behaviour based on the properties and assumptions of the transgenic modification and use the predictions to assess the likelihood of events - WHO (2)

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