Guidance Framework for Testing of Genetically Modified Mosquitoes

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Glossary

Alleles – different forms of the same gene.

Area-wide control – Methods of reducing pest damage whose effectiveness depends on application over large expanses. This contrasts particularly with personal protection, as provided for example by bed nets and repellents.

Biosafety committee – group responsible for implementing policies and guidelines related to use of potentially hazardous biological agents, including but not limited to infectious agents, human materials, and recombinant DNA studies. This group ensures that research involving these agents does not endanger researchers, laboratory workers, human research subjects, the public or the environment.

Cartagena Protocol on Biosafety - an international agreement dealing with safe handling, transport and use of living modified organisms resulting from modern biotechnology. See http://bch.cbd.int/protocol/

Clinical disease incidence - the number of new clinical cases per unit time for the at-risk population. This is typically determined by voluntary reporting of symptoms or community-based active case detection followed by a laboratory diagnosis test.

Cluster randomized trials – trials that group individuals into clusters, such as residents of particular villages or urban neighborhoods. Each cluster is randomly assigned an experimental treatment such as a placebo or drug, or, in the case of GMM, releases may be performed in one set of clusters and not in another.

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 stakeholder 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 for escape and/ or ecological confinement by geographic/spatial and/or climatic isolation.

Declaration of Helsinki - a set of ethical principles for the medical community regarding human experimentation, issued by the World Medical Association.

Deployment – implementation of GMM technology as part of a national or regional program for vector control.

Drive (also called gene drive) – a mechanism that increases the transmission of the transgene in a population above that which would be expected based on Mendelian inheritance. The increase is reflected in the excess proportion of progeny that carry the transgene.

Ecosystem - a biological system composed of a community of organisms and the nonliving environment with which it interacts

Endemic – a situation in which disease is present continuously in an area at some level.

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.

Epidemic - an increase in incidence and prevalence of disease affecting many people rapidly and extensively and above normal levels in an area, but not continuously present at such levels.

Ethics – an activity or inquiry intended to shed light on the correctness or justifiability of a given course of conduct.

Ethics committee (also called institutional ethics committee, institutional review board or ethical review board) – a group charged with providing oversight for biomedical and behavioral research involving humans, with the aim to protect the rights and welfare of research subjects.

Ethical review board – see *Ethics committee*

Fitness - description of the ability to both survive and reproduce and is 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, the alleles with higher fitness become more common.

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

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

Genetically engineered mosquitoes – see *Genetically modified mosquitoes*

Genetically modified mosquitoes (GMM; also called genetically engineered mosquitoes, transgenic mosquitoes, or living modified mosquitoes) - 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 – see also *Genetically Modified Organism*. GMM likely also will 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) – 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 different from the natural population, these are excluded from this definition.

Genotype – the genetic constitution of an organism.

Good Clinical Practice (GCP) - an international quality standard for trials involving human subjects, including protection of human rights, assurance of safety and efficacy and standards on conduct of clinical trials. See http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf

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.

Infection incidence - the rate at which new infections occur during the specific period of time.

Informed consent – the process intended to ensure that human subjects who will be observed or involved in a research activity are fully and explicitly advised of all risks, costs or inconveniences they may bear as a result of participating as a research subject, and voluntarily agree to accept or bear those risks and costs.

Institutional ethics committee (IEC) – see Ethics committee

Institutional review board (IRB) – see Ethics committee

Integrated vector management (IVM) - a rational decision-making process for the effective and efficient use of a combination of available resources in the management of vector populations, so as to reduce or interrupt transmission of vector-borne diseases. See: http://www.who.int/malaria/vector_control/ivm/en/

Living modified mosquitoes – see *Genetically modified mosquitoes*

Mark-release-recapture – a method used to estimate population size of free-living animals, including mosquitoes, and to study population survival and dispersal in space and time. A portion of the mosquito population under study is captured, marked (usually with fluorescent powders) and released. A portion of the population into which they were released is captured later and the number of marked mosquitoes within the sample is counted. The proportion of marked mosquitoes in the second sample allows estimation of the total number of animals in the whole population.

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

Nuremberg Code- an ethics code that serves as a basis for bioethical principles ensuring the rights of human subjects in medical research.

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.

Pharmacovigilence - the process of collecting, monitoring, researching, assessing and evaluating information on the long term adverse effects of medicines.

Phenotype – the observable characteristics of an organism, based on genetic and environmental influences.

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

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.

Prevalence of infection - the frequency of infection within a population at any given time.

Refractoriness – a condition in which the mosquito is intrinsically unable to support the development of a pathogen to an infective stage or to a point of sufficient abundance such that the mosquito cannot transmit disease.

Regulation - an official rule to manage the conduct of those to whom it applies, usually developed from legal interpretations of legislation and implemented by government ministries or agencies.

Regulatory agency (also called regulatory authority, ministry, regulatory body, or regulator) - a public authority or government entity responsible for exercising authority over some area of activity in a supervisory capacity.

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 comprised of risk identification, risk assessment, risk management and risk communication.

Risk assessment – a scientific and systematic description of the level of risk for any hazard.

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

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

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

Self-sustaining (also called self-propagating) – GMM approaches where the heritable modification is intended to spread and be maintained indefinitely through the target population.

Sterile insect technique (SIT) - the inundative release of factory-produced sexually-sterile insects into wild native insect populations. 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 release of both sexes has also been effective. SIT has been applied most widely against agricultural pests.

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 mosquitoes – see *Genetically modified mosquitoes*

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

Acronyms

ACRE: UK Advisory Committee on Releases to the Environment
AHPA: USA Animal Health Protection Act
APHIS: US Animal and Plant Health Inspection Service
AU: African Union
BCH: Biosafety Clearing-House of Cartagena Protocol on Biosafety
BIRC: Biosafety Information Resource Centre of Convention of Biological Diversity
BRS: USDA, APHIS, Biotechnology Regulatory Services
CBD: Convention of Biological Diversity
CDC: USA Centers for Disease Control and Prevention
CIBIOGEM: Interministerial Commission on Biosecurity and Genetically Modified Organisms in Mexico
CONABIO: National Commission on the Use and Knowledge of Biodiversity of Mexico
CPB: Cartagena Protocol on Biosafety
CVPA: Singapore Control of Vectors and Pesticides Act
DDBIA: Malaysian Destruction of Disease-Bearing Insects Act
DEFRA: UK Department for Environment, Food, and Rural Affairs
DHHS: US Department of Health and Human Services
DHS: US Department of Homeland Security
EA: Environmental Assessment under the US National Environmental Policy Act
EFSA: European Food Safety Authority
EIA: Environmental Impact Assessment (also known as a Strategic Environmental Assessment or Environmental Impact Statement)
EIS: Environmental Impact Statement under the US National Environmental Policy Act
EPA: US Environmental Protection Agency
EPHA: Singapore Environmental Public Health Act
ERA: Environmental Risk Assessment
ESA: US Endangered Species Act
EU Directive 2001/18/EC: Deliberate Release Directive
EU: European Union
FAO: Food and Agriculture Organization of the United Nations
FFDCA: US Federal Food Drug and Cosmetic Act
FIFRA: USA Federal Insecticide and Rodenticide Act
IAEA: International Atomic Energy Agency
IAPSC: Inter-African Phytosanitary Council of the African Union
IAS: Invasive Alien Species
IBC: Institutional Biosafety Committee
ICCPR: International Covenant on Civil and Political Rights
ICESCR: International Covenant on Economic, Social and Cultural Rights
IDA: Singapore Infectious Diseases Act
IEC: Institutional Ethics Committees
IHR: International Health Regulations
IIBC: International Institute of Biological Control (now CABI)
IITA: International Institute of Tropical Agriculture
IOBC: International Organization for Biological Control
IPLMO: International Project on LMO Environmental Risk Assessment Methodologies Project
IPPC: International Plant Protection Convention

ISPM: International Standards for Phytosanitary Measures
IVM: Integrated Vector Management
LMO: Living modified organism
NAPPO: North American Plant Protection Organization
NBSAPs: National Biodiversity Strategies and Action Plans of the Convention of Biological Diversity
NEPA: US National Environmental Policy Act
NGO: Non-governmental organization
NPPO: National Plant Protection Organizations
NSAR: The USA National Select Agents Registry
NSF: US National Science Foundation
OSHA: US, Occupational Safety and Health Administration
OIE: World Organisation for Animal Health (formerly Office International des Epizooties)
PFOA: Problem Formulation and Options Assessment Handbook
PMN: US, EPA premanufacturing notification
PPA: US Plant Protection Act
PRA: Pest Risk Analysis
SPS Agreement: WTO Agreement on the Application of Sanitary and Phytosanitary Measures
TSCA: US Toxic Substances Control Act
UDHR: Universal Declaration of Human Rights
UNDP: United Nations Development Programme
USDA: United States Department of Agriculture
VS: USDA, APHIS, Veterinary Service
WHO: World Health Organization
WMA: World Medical Association
WTO: World Trade Organization

Foreword

Vector-borne diseases are endemic in more than 100 countries and affect approximately half of the world's population. Many types of arthropods may serve as disease vectors, but this guidance focuses particularly on mosquitoes. Mosquitoes transmit several diseases of major global public health importance, including malaria and dengue fever.

Despite ongoing and intensive control efforts, malaria and dengue continue to exact a huge public health toll. Estimates of malaria-related deaths in 2010 range from 655,000 (World Malaria Report, 2011[1]) to over 1.2 million (Murray *et al*, 2012), with the majority of deaths occurring among African children under 5 years of age. 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 countries."[2] Yet it is acknowledged widely that this goal will not be met without new tools (Greenwood *et al,* 2008; Mendis *et al*, 2009; Alonso *et al*, 2011). An estimated 2.5 billion people live in areas where dengue viruses can be transmitted. It is believed that 50 to 100 million infections occur annually, resulting in approximately 500,000 cases of severe dengue requiring hospitalization (WHO Fact Sheet No. 117: Dengue and Severe Dengue[3]). Dengue is resurging in the Americas and Asia/Pacific regions, influenced by factors such as increased urbanization, climate change, and conflicting control priorities (Anonymous, 2008). In response, the Pan-American Health Organization and its member states developed a hemispheric action plan for eventual eradication of *Aedes aegypti* , the main vector of dengue, in the Americas (Anonymous, 1997; Anonymous, 1998). However, dengue continues to plague countries in Latin America, Asia and Africa, and the disease is now recognized as one of the most common reasons for hospital admission in Asia and the Americas during the rainy seasons (Whitehorn and Farrar, 2011).

Attacking 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 successful elimination of malaria transmission in the U.S. and Italy in the early 20th century (Kitron & Spielman, 1989) and dengue in the Americas in the early 1960's (Pinheiro & Corber, 1997). Vector-targeted approaches remain a mainstay of current disease-control practices. However, given the magnitude of ongoing malaria and dengue incidence, current efforts clearly are insufficient to meet the need. Moreover, dependence on a limited number of insecticides for vector control increases the risk that mosquitoes will develop resistance, as is now being widely reported (Butler, 2011). In considering the potential of new technologies to address the unmet needs of mosquito control, it is necessary to evaluate the benefits and risks in the context of the current situation. The potential public health benefit of practical and effective new tools to reduce or even eradicate diseases such as malaria and dengue is clear and widely recognized. The risk incurred by testing new and unproven strategies must be evaluated against the risks to human health and the environment posed by maintaining the *status quo*, which include ongoing disease and use of broad spectrum insecticides.

[1] http://www.who.int/malaria/world_malaria_report_2011/WMR2011_factsheet.pdf
[2] http://www.rollbackmalaria.org/rbmvision.html
[3] http://www.who.int/mediacentre/factsheets/fs117/en/

For more than two decades, scientists have been working to harness the promise of molecular biology to develop genetically modified mosquitoes for use as public health tools to prevent the transmission of these diseases. Several of these genetic technologies are now advancing to field testing. The introduction of molecular biology techniques represents the next step in a progression that builds on the widespread success of programs employing release of radiation-sterilized insects to control the Mediterranean fruit fly (med fly) and other insect pests affecting plants and animals, a process known as Sterile Insect Technique (Dyck *et al*., 2005). Radiation- and chemo-sterilization methods also have been applied to mosquitoes (Dame *et al*., 2009), but they pose several difficulties that might be overcome using genetic modification technologies. Recent advances in the development of genetically modified mosquitoes have raised hopes for the availability of new, potent and cost-effective tools to aid in the fight against malaria and dengue. However, concerns have also been raised about the need for thorough, thoughtful and transparent preparation for and conduct of field trials (Reeves *et al*, 2012).

Since 2001, scientists involved in this research have, with the support of The UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO-TDR) and other funders, gathered periodically to consider the requirements for testing and implementation of genetically modified vectors. Through such discussions, broad agreement has been reached within the scientific community on two tenets, which thus far have been adhered to:

- First, field testing should begin with release of sterile or otherwise self-limiting modified male mosquitoes in order to gain experience with the technology under circumstances where its effects can be reversed by halting releases (Benedict and Robinson 2003). Field releases of genetically-modified mosquitoes 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 (Alphey *et al.*, 2002; Benedict *et al.*, 2008). No genetically-modified mosquitoes designed to replicate and spread the modification to wild-type mosquitoes have yet been tested outside of the laboratory.

As the research progresses, a need has been expressed both within the scientific community and by the public for additional standards and guidance. WHO-TDR and the Foundation for the National Institutes of Health (FNIH) co-sponsored a technical consultation meeting in 2009 to assess current progress and future development of genetically-modified mosquito technologies. The meeting was attended by participants from around the world with expertise in molecular biology, medical entomology, ecology, regulatory requirements, ethical, social and cultural issues, as well as staff from WHO, FNIH and other research funders.[4] Participants recommended the establishment by WHO and FNIH of a working group to develop a guidance framework to provide quality standards for assessing the safety and efficacy of genetically modified mosquitoes and addressing legal, ethical, social and cultural issues that arise during their development and deployment. A multidisciplinary effort was commissioned and over 40 experts recruited to contribute to the working groups or serve as external reviewers. In accordance with the recommendations, the group included many members who possessed a broad knowledge in their topic areas but were not involved directly in research on genetically modified mosquitoes.

[4] http://apps.who.int/tdr/svc/publications/training-guideline-publications/gmm-report

Because of the breadth of different genetic approaches that are under consideration and conditions under which they might be used, it is not possible to provide an exact formula for evaluation of all genetically modified mosquito technologies. It will be necessary to determine the specific needs on a case-by-case basis. Rather, the guidance framework presented here proposes standards of efficacy and safety testing that are complementary to those used for trials of other new public health tools, including drugs, vaccines and insecticides, drawing also from relevant experience in agriculture and biocontrol. It examines the fundamental considerations for addressing public engagement and transparency needs in research on genetically modified mosquitoes, taking into account lessons learned from previous introductions of new technologies in the fields of health and agriculture. Finally, it reviews existing regulatory requirements and guidance that are either directly pertinent to research on genetically modified mosquitoes or may provide precedents and examples for establishing the appropriate level of oversight. This *Guidance Framework for Testing of Genetically Modified Mosquitoes* is intended to foster quality and consistency in the processes for testing and regulating new genetic technologies. This will contribute to comparability of results and credibility of conclusions in addressing the requirements for decision-making by countries interested in potential use of these technologies as public health tools for control of vector-borne diseases.

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Key Messages

Foreword and Introduction

1. Despite ongoing control efforts, diseases transmitted by mosquitoes, such as malaria and dengue, continue to pose an enormous global health burden. Multinational public health organizations have called for the eradication of malaria and of the major mosquito vector of dengue. There is broad recognition of the need for improved tools to combat these diseases.

2. Currently available methods to control mosquito vectors of malaria and dengue are based on use of insecticides and elimination of mosquito larval breeding sites. In considering the potential of new technologies to address the unmet needs of mosquito control, it is necessary to evaluate the risks and benefits in the context of the current situation. Thus, the risk incurred by testing new and unproven strategies should be assessed against the risks to human health and the environment posed by maintaining the *status quo*, which includes both ongoing disease and exposure to broad spectrum insecticides.

3. Genetically modified mosquitoes (GMM) have been proposed as a possible new tool to reduce transmission of malaria and dengue. This *Guidance Framework* is intended to foster quality and consistency of procedures for testing of GMM, which will contribute to comparability of results and credibility of conclusions in addressing the needs for decision-making by those considering the use of GMM as public health tools to control mosquito-borne diseases. The *Guidance Framework* should be useful to readers interested in:
   - What GMM technologies and applications currently are being contemplated;
   - What safety, efficacy, regulatory and social/ethical issues are involved in taking GMM from the laboratory to field testing;
   - What precedents exist for how these issues have been dealt with to date;
   - What existing regulatory frameworks and international agreements are relevant to GMM testing and eventual implementation.

4. GMM technologies currently under development are aimed at either reducing the size of the mosquito vector population to an extent that will significantly reduce pathogen transmission ("population suppression") or at reducing the inherent ability of individual mosquitoes to transmit a particular pathogen ("population replacement").

5. These technologies can be further defined according to how long the GMM are intended to persist in the environment following release. The persistence of the GMM effect will depend upon the transgene components and their behavior.

6. With "self-limiting" approaches, the genetic modification will decline in frequency within the mosquito population over time until it disappears. In some cases, the GMM are meant to be sterile and thus unable to pass the genetic modification on to future generations through mating. In other cases, the GMM are meant to mate and introduce the effect briefly into the local mosquito population, but the modification will gradually be diluted out by crossing with local mosquitoes over a number of generations until it is lost. Thus, the effect of self-limiting approaches can only be maintained by periodic re-releases of GMM, and how often these releases must be performed will depend upon the type of genetic modification. From a risk assessment perspective, these

approaches can be reversed by halting releases and therefore are unlikely to produce permanent changes in the environment; however, the need for frequent reintroductions is associated with ongoing costs of production and delivery.

7. With "self-sustaining" approaches, the genetic modification is intended to be spread into the local mosquito population and to persist indefinitely. These approaches have the potential to provide highly durable and cost-effective protection against pathogen transmission, but any unforeseen effects may be more difficult to reverse than would be the case for self-limiting approaches.

8. Because of the breadth of different genetic approaches that are under consideration and conditions under which they might be used, it is not possible to provide a universal formula for evaluation of these technologies. It will be necessary to determine the specific needs on a case-by-case basis, keeping in mind both the potential benefits as well as risks.

9. GMM technologies offer several theoretical advantages over conventional vector control strategies. They can reach mosquito populations and mosquito larval breeding sites that have traditionally been hardest and most expensive to access, by exploiting the natural behavior of mosquitoes to mate and seek sites for egg-laying. For example, GMM are well-suited to urban settings, where current control measures are largely ineffective due to the wide availability of cryptic mosquito larval breeding sites. Additionally, GMM can reach outdoor and day-biting mosquitoes that often escape control methods such as bed nets and indoor insecticide spraying. The modification can be made specific for the target mosquito species, and thus avoid ecological and environmental hazards associated with commonly used broad-spectrum insecticides. GMM could provide continuous protection in situations where other disease control methods have been interrupted, and prevent the reintroduction of the pathogen after successful elimination efforts. It is important also to note that GMM technologies are compatible with other disease control methods and could be incorporated into integrated vector management programs.

10. A phased testing pathway is recommended for GMM, analogous to the development pathway for other new public health tools, with systematic assessment of safety and efficacy at each step. New GMM technologies would first move from the laboratory (Phase 1) to testing under confined conditions that provide a more natural setting but still limit release into the environment (Phase 2). Phase 2 may involve testing under physical confinement, as in a large cage equipped to simulate a disease-endemic setting, or under ecological confinement, as under geographic, spatial or climatic isolation. The plan for confined testing will be informed by risk assessment. Following such confined testing, GMM may proceed to a series of staged open release trials in Phase 3, designed to measure performance under different conditions and to assess the ability of GMM to reduce infection and/or disease in human populations. Based on results from Phase 3, a decision may be made to deploy GMM as a public health intervention (Phase 4). Phase 4 would be accompanied by long term monitoring of safety and efficacy. The transition from one phase to the next will be subject to "go/no-go" decision criteria, including efficacy and safety endpoints, regulatory and ethical approvals, and social acceptance.

11. The critical path for GMM development will include not only proof of efficacy, but also proof of acceptability and deliverability. Risk analysis, community and stakeholder engagement, and regulatory approval contribute to proof of acceptability. Deliverability will require an operating model with appropriate prospects for financing to support deployment and subsequent monitoring, sufficient technical and production capacity, quality control processes, capability to provide

necessary mitigation in the case of unforeseen effects, as well as commitment to ongoing stakeholder engagement.

Efficacy Evaluation

12. GMMs must be effective in reducing transmission of the targeted pathogen(s) and not detrimental to the environment and human health if they are used as public health intervention tools. Demonstration of efficacy will be a critical determinant for decision-making about deployment.

13. The efficacy of GMM may be measured by both entomological and epidemiological endpoints. The entomological endpoint is a reduction in the risk of disease transmission as measured by specific mosquito population characteristics. The epidemiological endpoint is a reduction in the incidence of infection or disease in human populations. Whereas entomological endpoints may be relevant through all phases of testing, epidemiologic endpoints will probably only become significant in Phase 3.

14. The most direct measure of entomological endpoint is a reduction in the estimated transmission intensity, called the Entomological Inoculation Rate (EIR). Because measuring EIR reductions is difficult or impossible during Phase 1 and Phase 2, it is necessary to infer reductions in EIR by surrogate vector indicators that would contribute to the EIR, such as vector population size, transgene frequency, GMM fitness, or pathogen replication within the vector.

15. A powerful design for determining efficacy of GMM applications is the Cluster Randomized Trial. Such trials must be designed to allow measurable reductions in infection incidence. Careful site selection is necessary to increase the likelihood of detecting significant results. The influence of seasonal and inter-annual variations and spatial heterogeneity in incidence on trial design must be considered. “Go” and “no-go” criteria for moving forward should be determined. Independent verification of results should be considered.

16. GMM likely will be applied in the context of conventional control measures. Thus, the effect of other on-going control measures on the outcomes of the GMM trials must be considered in the trial design. The efficiency of GMM relative to conventional control will in part determine their utility.

Biosafety

17. Risk is the likelihood that harm will occur from a particular action. The level of risk is estimated as the product of the expected probability that a harmful event will occur and the expected consequences, or impact, of the event.

18. Risk assessment is a methodological approach to systematically define the level of risk. Risk management encompasses strategies developed to avoid and reduce risk to acceptable levels. Risk analysis encompasses risk assessment and risk management, as well as risk awareness and risk communication, which inform the concerns on which to focus and the acceptability of risks, and convey the results of these processes to the public and to decision-makers.

19. The evaluation of risk should be set against the benefits of GMM for improving human health. Benefit-cost analyses provide the framework under which the appropriate (economic, health, social) returns of a GMM release program can be quantified, and provide a context for decision-making about the level of acceptable risk. Risk assessment of novel technologies should be set against the risk of relevant alternatives, such as the risk of no action or the risk of conventional control methods. "Causes more harm" than current practice is a reasonable comparator for risk assessment of GMM.

20. The core functions of risk analysis are assessment and management. Risk assessment should determine (1) a characterization of events leading to potential negative impacts of the GMM, i.e. a list of defined hazards; (2) the level of exposure to these events leading to quantification of the likelihood and consequences of them affecting target organisms, non-target organisms and human health; (3) the levels of uncertainty associated with the potential events, levels of exposure, and their consequences. Risk management should (4) evaluate proportionate measures that are needed to mitigate any harm or uncertainty; and (5) demonstrate how management measures would render the identified risks as acceptable.

21. Risk assessment should follow and be proportionate to the phased testing pathway of GMM. Quantitative and qualitative risk analysis frameworks allow a formal way in which to assess, manage and communicate appropriately the identified risks. Once risk is assessed, appropriate risk management strategies may be devised.

22. On evaluation, risk in some cases may be judged as negligible, as when the probability a harmful event will occur is determined to be very low or the consequences of it occurring would be minimal. Moreover, in many cases, despite potentially harmful events being identified, the practical level of risk to which the public is exposed can be reduced to acceptable levels by effective management. The identification of potential hazards does not in itself indicate an unacceptable risk.

23. Biosafety considerations in Phase 1 testing of GMM should include:
    - how appropriate comparators will be chosen, what appropriate comparisons should be made, and what endpoints will be used for these comparisons of risk;
    - stability and effectiveness of the transgene at the population-level and the consequences of incomplete or partial transgene function;
    - the phenotype of GMM with multiple transgenes, rather than the effect of individual genes;
    - the methodology for and impact of sex separation, if appropriate to the GMM technology being assessed;
    - how GMMs will be discriminated within a wild population after release, how the maintenance of gene integrity will be monitored, and how trial endpoints will be determined;
    - the type, strength and function of the appropriate ecological processes affecting the GMM population;
    - appropriate ecological and biological comparisons for non-target organisms.

24. Additional biosafety considerations in Phase 2 testing should include:
    - determination of the need for physically confined testing prior to ecologically confined testing;
    - appropriate site selection criteria for ecologically confined trials, bearing in mind the spatial location, timing and duration of confined field trials;

- basic ecological, entomological and epidemiological information necessary to evaluate the efficacy of confined field trials;
- appropriate information on the ecological processes critical to the evaluation, efficacy and success of the GMM;
- spatial extent of the trial, including potential risks in areas outside the designated trial site(s);
- development of detailed Standard Operating Procedures (SOP) to ensure that rearing, release and monitoring are carried out consistent with the relevant assumptions made in risk assessment, with clear lines of responsibility and reporting, and risk management strategies for field trials;
- potential for unanticipated effects on disease burden;
- non-target species assessments, as applicable to confined field trials.

25. Additional biosafety considerations in Phase 3 testing should include:
    - characterization of local target mosquito ecology as required to set appropriate trial endpoints, including impact on human health and the wider environment;
    - methods for evaluating GMM success through population-level assessments;
    - appropriate risk management plans for any potential resistance to the genetic modification, designating the lines of responsibility for managing this risk;
    - proportionate assessment and management of non-target and off-target effects and the likely risk of transgenic gene flow;
    - proportionate assessment and management of risks associated with the mass production of mosquitoes.

26. If and when a decision is made to deploy GMM broadly as a public health tool, there will be an ongoing need for post-implementation quality control and surveillance to monitor safety and effectiveness under full operational conditions. Biosafety considerations in Phase 4 should include:
    - methods available for ongoing monitoring of the epidemiologic impact of GMM on human health;
    - methods available for ongoing monitoring of safety for the environment and human health (in a manner analogous to the pharmacovigilence that applies to medicines for preventing or intervening against disease);
    - available mitigation methods in the case that a negative effect is observed;
    - risk implications and management of the movement of GMMs across borders.

27. Independent ongoing safety review is recommended, covering relevant aspects of environmental monitoring and human health. This may be accomplished through existing institutional or national level biosafety committees or establishment of new review bodies focused on GMM activities. Strengthening of biosafety oversight capabilities within disease endemic countries should be encouraged. National biosafety laws developed for GM plants may need to be reinterpreted for GMM, or additional guidance provided.

Ethics and Engagement

28. In the design of GMM trials, a key set of questions relates to the ethical implications, including the nature and scope of the obligation to respect host communities and what type of protections should

be provided to them. Respect for communities should be understood as an overarching ethical goal within GMM trials.

29. Although activities of ethical reflection and engagement often overlap with those of regulatory compliance, ethical issues and responsibilities are generally broader than just those activities specifically mandated by administrative law or organizational policies. It should not be assumed that regulatory compliance implies that ethical and engagement responsibilities have been addressed adequately.

30. Democratic governance of technology requires that proposals such as testing of GMM be openly discussed and debated in a manner that receives the attention of scientists and decision makers, and in such a way that participants' voices can be heard.

31. The ethics and engagement component of a GMM research program will take place at multiple levels, including:
    - Within the project team – Team members and their advisors should articulate the value and social purpose of the research, engage in ongoing and structured ethical reflection (including consideration of dissenting opinions and legitimate public concerns), document publicly the ethics and engagement activities that have been done, and evaluate the performance of these activities. All of these efforts should feed into further development and refinement of plans and methods.
    - With the host community –Researchers have ethical responsibilities to people living within a trial site. For that subset of individuals classified as "human research subjects" according to standard regulatory criteria, informed consent obligations will apply. However, there may be many individuals living within a trial site who are not, in a traditional sense, subjects of the research at hand, but who nonetheless may be affected by the conduct of research. Community engagement addresses ethical obligations to these people, including undertaking procedures that would be expected to identify them, advise them that they may have interests at stake, find out what concerns they may have, respond to those concerns, and reach some form of agreement about whether the trial should proceed.
    - With third parties - Individuals *not* immediately associated with the trial site will take an interest in the conduct and outcome of the research, such as public health or international development organizations, other scientists, members of civil society organizations, the press, and the general public. The ethical obligation to third parties is not to seek them out proactively to ensure awareness of the research, but to consider and respond to their expressed concerns and interests in a respectful manner. GMM projects should incorporate a communications/public engagement strategy that includes education about the goal and methods, but also provides opportunities for follow-up discussion.

32. Ethics and engagement activities should begin before Phase 1 proof-of-concept work has been completed. Adequate plans for communication and engagement should be put in place before even the earliest stages of field testing begin, in order to avoid the possibility of misunderstandings and miscommunications that could undermine respect for the host community and jeopardize future research. Plans should include initiating interactions with policy-makers to explain research goals and develop an open dialog.

33. A need for community engagement and authorization activities will arise in Phase 2 of the GMM testing pathway. Before proceeding to confined release trials, plans should be developed for

responding to ethical obligations to individuals being asked to participate as human research subjects and/or to communities being asked to host trials. Communications should explain that trials are research activities intended to test the efficacy of a new technology, a protective effect is not assured, and the community must continue to employ other available methods to protect themselves from disease transmission.

34. Community engagement and authorization activities will expand in Phase 3, and human subjects issues will become more prominent in trials undertaken to determine the epidemiologic impact of GMM.

35. In Phase 4, ethical responsibilities to those who are affected by the technology are increasingly likely to converge with established processes. Deployment of GMM will be a public health initiative taking place in the context of existing legal, regulatory and political institutions. However, the need for public engagement activities is likely to continue.

36. It will be important for members of the scientific team to be involved in ethics and engagement activities. However, many aspects of these activities also will require the specialized skills of social scientists and communications experts. Adequate funding for these activities will be imperative for the successful accomplishment of the research objectives.

37. A need can be anticipated for training of project scientists about research ethics, and for training of institutional or national ethics review committees in the specialized issues associated with vector biology research.

Regulatory Frameworks

38. Regulation is an enabling process that ensures safety and efficacy are consistent with social values. Regulation of GMMs may be encountered early in the research process and throughout development and implementation. Regulation can be expected at institutional, state, provincial, and national levels, all of which may have to be addressed simultaneously.

39. Each country has its own sovereign regulatory process, but overarching international agreements or treaties also may be relevant. Early investigation of the regulatory processes in a given country and open communication with the national officials, risk assessors, and decision makers are imperative in order to understand the requirements relevant to GMM.

40. Early interaction with regulators not only will serve to identify the appropriate regulatory pathway for GMM, but proactive communications also will help to build understanding within regulatory agencies about the GMM technology, as well as the goals and methodologies of the project. There may be a need to strengthen familiarity with entomology research methods and/or biosafety procedures, and this should be planned for accordingly.

41. The Cartagena Protocol on Biosafety is accepted by almost all developing countries and is anticipated to be an important influence on GMM regulatory processes and risk assessments. It will be essential to work with regulators to ensure understanding of the differences between GMM and GM plants or crops, including that human health benefits are relevant as part of the regulatory decision-making process for GMM. Limited resources available to GMM developers, especially

where products are intended primarily to serve the public health needs of developing countries, make it important for authorities to exercise discretion in imposing regulatory requirements, taking into account scientific rationale and relative risks.

42. Regulation of GMM in a country may present unanticipated costs and potential delays that must be recognized as early as possible. Plans for dealing with such contingencies should be put in place and suitably resourced.

43. Informed public involvement and consent in the GMM regulatory decision process is a necessity if implementation is to occur without adverse public reaction. Regulatory processes often include formal public consultation opportunities.

44. While there currently is no standardized procedure for addressing potential transboundary movement of GMM that are self-sustaining or with gene drive, some precedent is provided by prior introductions of classical biological control agents in agriculture. A regional notification and agreement process may be advisable for planned introductions capable of autonomous international movement beyond the scope of provisions in the Cartagena Protocol and may best involve a multilateral organization, such as the WHO, in a coordinating capacity.

1. Introduction

Summary : *The need for better methods to combat mosquito-borne diseases is widely recognized. Recent research offers the possibility that genetically modified mosquitoes (GMM) could be used to prevent pathogen transmission. GMM provide several theoretical advantages that make them attractive for vector control, such as specificity and the ability to function in areas that are difficult to reach with conventional control methods. Different GMM 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 so that GMM persist for only a brief period of time (self-limiting) or so that the modification is passed on through local wild mosquitoes and persists indefinitely within the local mosquito population (self-sustaining). Ongoing releases of self-limiting GMM will be required to maintain effectiveness. Self-limiting approaches may be attractive from an environmental safety perspective. Since they do not persist in the environment and cannot spread far beyond the release site, self-limiting approaches can be reversed by halting releases. However, self-sustaining approaches could ultimately provide more durable and cost-effective public health solutions. A phased testing pathway is recommended, in which new GMM 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. Once GMM are incorporated into national or regional vector control programs, case-specific monitoring of effectiveness and safety should be continued to ensure acceptable quality and performance standards and inform any necessary management responses.*

Current mosquito control efforts rely heavily on chemical methods including insecticide-treated bed nets, indoor residual spraying with insecticides, outdoor insecticide fogging, and application of chemical larvicides, or management of standing water for mosquito larval breeding sites. Despite diligent application of available control strategies, including improvements and expanded use of bed nets, mosquito-borne diseases such as dengue (Morens and Fauci 2008; WHO Fact Sheet No. 117[5]) and malaria (Breman *et al.*, 2007; World Malaria Report Fact Sheet[6] 2011, Murray *et al.*, 2012) continue to pose major global health challenges. WHO officials have stated that "global eradication of malaria cannot be expected with existing tools" due to the difficulties of interrupting transmission in sites with ongoing high vectorial capacities (Mendis *et al.*, 2009). Malaria mapping and modeling studies support this conclusion (Hay *et al.*, 2009, Griffin *et al.*, 2010). Similarly, a WHO-TDR-sponsored dengue scientific working group acknowledged that "we are collectively failing to meet the threat posed by dengue as the disease spreads unabated and almost 40% of the world's population now live at risk of contracting it" (Farrar *et al.,* 2007). Re-emergence in the Americas over the last two decades provides an example, where dengue is now exacting a substantial public health and economic toll (Shepard *et al.*, 2011). Limitations of current vector control methods include: inability to reach mosquito larval breeding sites and adult resting sites; evolution of resistance to chemical agents; compliance and infrastructure issues; concern about impact on the environment and/or toxicity to humans; and importantly, cost. The

[5] http://www.who.int/mediacentre/factsheets/fs117/en/
[6] http://www.who.int/malaria/world_malaria_report_2011/WMR2011_factsheet.pdf

ongoing costs of vector control are substantial[7], and maintaining the high levels of donor and national government support necessary to achieve high coverage of control measures over long periods of time has historically proven daunting (Mills *et al.*, 2008; Leach-Kemon *et al*., 2012). Thus, there is a recognized need for new, sustainable, and cost-effective vector control tools.

Intensive interest arose in the late 1980s for the application of modern genetic engineering technology to arthropod vectors as a useful approach for limiting transmission of human pathogens (Beaty *et al.*, 2009). Subsequent research has focused in large part on two high impact mosquito species, *Anopheles gambiae* and *Aedes aegypti*, which serve as major vectors for malaria and dengue, respectively.

Substantial progress has been made on challenges such as sequencing the genomes of these two important vector species, achieving stable germline transformation, identifying sex-, tissue- and stage-specific DNA control elements, identifying genes involved in susceptibility or resistance to infection/insecticides, and developing models for methods to spread heritable modifications into native mosquito populations within an epidemiologically-relevant timeframe as needed to achieve disease control. The initial technical objective, germline transformation, was accomplished in all major mosquito genera (Allen *et al.*, 2001; Catteruccia *et al.*, 2000; Jasinskiene *et al.*, 1998) and can be considered routine for several species. Beyond similar preliminary achievements, effector genes have been developed that accomplish proof of principle for either refractoriness or sterility. Examples include: 1) mosquitoes refractory to malaria parasites (Corby-Harris *et al.*, 2010, Isaacs *et al*., 2011, Ito *et al.*, 2002) and dengue virus (Travanty *et al.*, 2004; Franz *et al.*, 2006); and, 2) mosquitoes that are sterile (Windbichler *et al.*, 2008) or that function in a manner to limit reproductive potential (Fu *et al.*, 2010; Phuc *et al.*, 2007; Thomas *et al.*, 2000). Additional methods have been proposed or demonstrated that await development in transgenic mosquitoes (e.g. Marshall *et al.*, 2010; Papathanos *et al.*, 2009; Schliekelman and Gould, 2000). Efforts can also be envisioned to develop additional effectors to reduce life span or alter behaviors in a beneficial way.

Although much work remains to be done, it is now possible to envision a pathway toward realization of successful implementation of genetic technologies for the control of mosquito-borne diseases. A multidisciplinary effort will be required, encompassing not only additional scientific advances, but also complementary planning for ethically and environmentally-responsible testing as well as for reliable, cost-effective and socially-acceptable deployment. Consequently, the technical consultation on genetically modified mosquitoes (GMM) organized in May 2009 by WHO-TDR and FNIH recommended that a guidance framework document be developed for assessing safety and efficacy and addressing regulatory and ethical, social and cultural issues during the development and testing of GMM (WHO, 2009). The framework presented here is intended to provide a basis for conduct of trials according to best practices that will contribute to comparability of results and credibility of conclusions. This should facilitate decision-making by countries regarding the potential testing and use of GMM as public health tools for prevention and control of malaria, dengue and other mosquito-borne diseases.

GMM Technologies

Currently contemplated GMM technologies are designed to have two major types of effect:

[7] Global Malaria Action Plan, Table II.4 http://www.rollbackmalaria.org/gmap/2-5.html

- ***Population suppression*** *- strategies that target vector "demography" with the intent to reduce (suppress) the size of the mosquito population such that it would not be able to sustain pathogen transmission.* These include methods to reduce the overall numbers of female mosquitoes (with or without a concomitant direct effect on males), which will result in decreased reproduction. Examples of how this could be accomplished include biasing against the development of female progeny (sex-ratio distortion), reducing female fertility, or introducing a mechanism that incapacitates or kills young female mosquitoes. This category also includes methods to shorten the lifespan of female mosquitoes, thus decreasing the length of time available both to transmit a pathogen from one person to the next and to reproduce.

- ***Population replacement*** *- strategies that target vector competence with the intent to reduce the inherent ability of individual mosquitoes to transmit a given pathogen.* This involves the introduction of engineered DNA and/or the manipulation of endogenous genes so as to inhibit pathogen replication within the mosquitoes, making them refractory to transmission of particular viruses or parasites. Upon release into the environment, these refractory GMM will be expected to introduce, through mating, the change into the local mosquito population, "replacing" their ability to spread the targeted pathogen with a reduced or eliminated transmission capability.

These strategies can be further categorized according to the ability of GMM to persist following release (Table 1.1). This will depend largely on a combination of two characteristics. The first is "fitness cost" (a decrease in the mosquito's ability to survive and reproduce as a result of the genetic modification) and the second is "drive" (a mechanism to increase the frequency of effector genes in a population at a rate faster than would be expected through normal Mendelian inheritance). Two general approaches are being pursued:

- ***Self-limiting*** *–approaches in which the GMM are unable to pass the modification on indefinitely through subsequent generations.* Self-limiting approaches are designed to impose a significant fitness cost, which will cause the GMM to decline in frequency over time until they disappear within the local population unless they are maintained by periodic new releases. In general, the greater the fitness penalty, the shorter the time period that the GMM would be expected to maintain their effectiveness. Indeed, a subset of the self-limiting approach is comprised of GMM that are sterile, or effectively sterile in that no viable adult progeny are produced from mating, and are thus unable to pass on the genetic modification to another generation. Other self-limiting approaches impose a less severe fitness cost, and therefore the modification will disappear more gradually from a population when releases stop. Some of these are designed to have a transient gene drive system that breaks down over time, at which point harmful effects on fitness predominate and the modification is expected to disappear from the population without recurrent releases. Thus, with self-limiting approaches, the combined effect of the fitness cost, which works against persistence, and drive, which promotes persistence, will dictate how long the GMM remain effective in the field and how often additional releases will be required.

A spectrum of different self-limiting approaches is under development. Some are being constructed to function similarly to the Sterile Insect Technique (SIT) that has been used successfully against pest insects affecting livestock and crops (Lindquist *et al*., 1992; Dyck *et al.,* 2005). In this case, few, if any, viable offspring are expected to result from the mating of GMM with native mosquitoes. The reproductive potential of the local population therefore will be reduced, resulting in population suppression. Such approaches will require frequent inundative releases of GMM to maintain effectiveness. With self-limiting approaches at the other end of the spectrum, i.e. those

which impose a lower fitness cost and incorporate weak drive, GMM from an initial release are expected to mate productively with local mosquitoes and introduce the desired effect into the population. However, the modification will gradually be diluted out over a number of generations of crossing with native mosquitoes until it is lost. Less frequent releases, involving lower numbers of GMM, would be required to maintain the effectiveness of this type of self-limiting approach.

Computer simulations indicate the potential for self-limiting approaches to substantially reduce vector-borne diseases (e.g. Atkinson *et al*., 2007). Moreover, it has been argued by some that release of self-limiting constructs should constitute the early stages of field testing in order to gain experience with GMM technology under circumstances where its effects could be withdrawn by halting releases (Benedict and Robinson 2003).

- ***Self-sustaining*** *– approaches in which heritable modifications are intended to spread indefinitely through the target population.* Self-sustaining approaches must be able to spread the effector mechanism into native mosquito populations within an epidemiologically-relevant timeframe. Thus, they require a strong drive mechanism capable of overcoming any fitness costs and increasing rapidly the frequency of the effector gene(s) from low initial levels to fixation, or near fixation. Once established, self-sustaining approaches are expected to be relatively stable and to require smaller and infrequent inoculative releases to maintain effectiveness. In the case of population replacement, the modification may become fixed permanently within the local population. With self-sustaining population suppression strategies, the modification may spread until the local vector population is greatly reduced or eventually eliminated. Computer simulations show that self-sustaining approaches have the potential to provide complete elimination of the disease pathogen in some circumstances, potentially replacing existing control methods (e.g. Deredec *et al*., 2012).

Table 1.1. GMM technologies currently under development

Strategy	**Approach**	
	Self-limiting	Self-sustaining
Population Suppression	- Modification reduces the number of progeny - Possesses either no gene drive or weak drive that will pass the modification through only a limited number of generations - Will not persist in the absence of continued releases	- Modification reduces the number of progeny - Possesses strong gene drive - Will spread the modification indefinitely or until the mosquito population is eliminated
Population Replacement	- Modification limits pathogen replication, thereby reducing transmission - Possesses weak gene drive that will pass the modification through only a limited number of generations - Will persist only until diluted out of the population	- Modification limits pathogen replication, thereby reducing transmission - Possesses strong gene drive - Will spread the modification through the population indefinitely

Characteristics of GMM

GMM technologies have certain favorable design characteristics as new vector control tools:

- They offer area-wide protection that is accessible to everyone, regardless of their socioeconomic level, and they do not require people to change their behavior in order to be effective.
- They do not require application of a chemical that must come into direct physical contact with the mosquito to be effective.
- They can reach mosquito populations and their larval breeding sites that have been traditionally the hardest and most expensive to reach using conventional vector control strategies by exploiting the natural seeking behavior of the mosquitoes to find mates and oviposition sites. This would include outdoor and/or day-biting vectors that escape control by bed nets and indoor spraying but may play an important role in transmission.
- A high level of specificity and stability reduces ecological, environmental and human health hazards associated with currently available broad spectrum insecticides.
- They are well-suited to application in urban environments where current control measures largely have proven inadequate.
- Technologies aimed at population suppression could reduce transmission of all pathogens transmitted by the same vector mosquito. For example, suppression of *Aedes aegypti* vectors could reduce transmission of dengue, yellow fever and chikungunya viruses.

Self-sustaining approaches have additional characteristics that would be useful in disease elimination or eradication efforts:

- Limited need for reapplication minimizes the requirement for ongoing mass production and delivery, which should make their use relatively inexpensive.
- Durability of activity should maintain effectiveness even in situations where other disease control methods must be temporarily suspended, as, for example, due to adverse weather conditions or civil unrest.
- Population replacement technologies reduce or eliminate the pathogen, rather than a particular mosquito vector. By not leaving an empty ecological niche, their effects are not limited by the potential for invasion of the treated area by other competent vectors.
- Some of the technologies could affect more than one local vector species if cross-mating occurs even at low levels, thus having the potential to reduce disease in regions where it is transmitted by related species.

Potential Utility of GMM

GMM primarily are being developed for use within disease endemic or epidemic situations as part of an area-wide control program to reduce the rate of pathogen transmission. GMM are likely to be used as part of an integrated approach, in conjunction with other disease control methods. Importantly, GMM-mediated methods to reduce the force of disease transmission by reducing the number of infectious bites could improve the protective potential of new vaccines. For example, modeling suggests that a pre-erythrocytic malaria vaccine will be much more effective in low transmission settings than in high transmission settings (Penny et al., 2008). Likewise, concurrent use of a vaccine would reduce the possibility that prolonged lack of pathogen exposure due to effective transmission control might result in loss of immunity within the human population (Ghani et al., 2009).

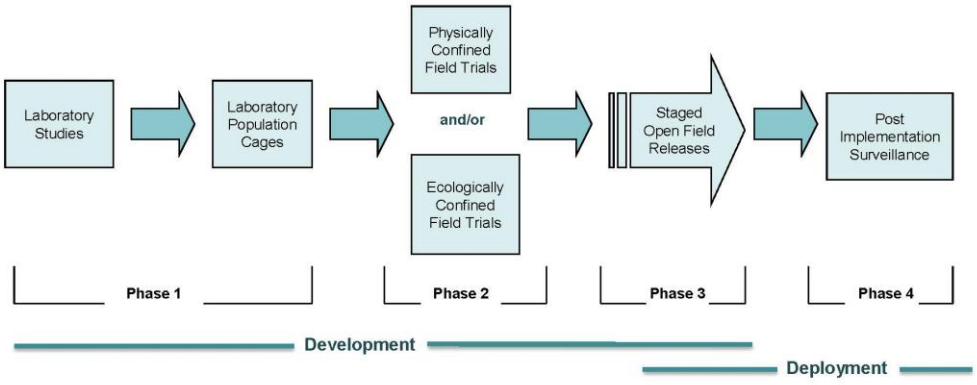
Because they would not require a high level of individual participation, GMM may not be as susceptible to the lack of compliance that is sometimes seen with conventional control programs after disease rates fall and the perceived threat is low. Ongoing area-wide protection provided by GMM, especially those that are self-sustaining, could prevent the reintroduction of the pathogen into the population (for example, by immigration of infected persons or mosquitoes) after successful regional elimination efforts. This may provide a valuable tool for disease eradication.

GMM technologies could also be useful as a preventative measure in regions where disease is not yet occurring. For example, where introduction of exotic mosquitoes species may take place, GMM could help to prevent their establishment. This is analogous to current utilization of SIT to prevent Mediterranean fruit fly infestation in otherwise pest-free areas.

GMM Testing Pathway

A series of workshops held in London and Atlanta in 2001 (Alphey *et al.*, 2002), Wageningen in 2002[8], and Nairobi in 2004,[9] began a process to discuss requirements related to the testing and implementation of genetically modified vectors. The concept of phased testing was advocated widely. The recommendation to develop a phased testing pathway was reiterated at a technical consultation, held at Geneva in May, 2009, which focused on practical and technical issues associated with moving new GMM technologies from the laboratory to field testing (WHO, 2009).

In accordance with these earlier recommendations, a stepwise testing process as illustrated in Figure 1 is proposed in this guidance framework. Subsequent sections expand upon specific considerations related to efficacy testing, safety testing, ethical, social and cultural issues, and regulatory decisions to be addressed at each phase.



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In **Phase 1**, efficacy and safety testing is anticipated to begin with small-scale laboratory studies, followed by testing in larger population cages in a laboratory setting conducted under appropriate containment facilities and procedures.[10] Laboratory testing under highly controlled conditions will allow preliminary assessment of whether the GMM demonstrate the desired biological and functional characteristics, with an eye toward future efficacy and safety.

For those GMM showing promise in Phase 1, **Phase 2** initiates confined testing in a more natural setting but under conditions that will limit release into the environment. Phase 2 may involve testing under physical confinement (sometimes termed "containment") within a large cage that simulates the disease-endemic setting while minimizing the possibility for escape. Testing under physical confinement, such as within a green- or screen-house type facility, has been advocated by experts for use in the early stages of testing of mosquitoes incorporating gene drive (Alphey *et al.*, 2002; Scott *et al.,* 2002, Benedict *et al.*, 2008). Phase 2 testing also may involve small-scale ecologically-confined field release. Ecological confinement entails geographic/spatial and/or climatic isolation that will limit spread of GMM into the environment. The decision about requirements for one or both components of Phase 2 testing will depend on the nature of the GMM technology, prior knowledge of its effects in other environments and other factors that are taken into account in the process of risk assessment (Section 3: Biosafety). Phase 2 trials will continue assessment of biological and functional activity of GMM, including their effect on local/wild-type mosquitoes, but because of their limited scale will only rarely provide information on the disease impact of the technology. Moving on to initiation of GMM trials in the environment and in disease-endemic countries will require thoughtful consideration and application of relevant ethical and regulatory practices (Sections 4: Ethics and Public Engagement, Section 5: Regulatory Frameworks).

Contingent upon satisfactory results of confined testing in Phase 2, the GMM technology may proceed to staged open release trials under **Phase 3**. This likely 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 transmission, or presence of other disease vectors in the region. While measurement of entomologic parameters is likely to remain the focus of early Phase 3 trials, later trials in this phase may include measurement of the impact of GMM on infection and/or disease in human populations. Trials to show epidemiological impact must be designed accordingly, with considerable thought toward the needs for achieving a statistically meaningful result. Although still focused on intense examination of the function and efficacy of GMM, Phase 3 trials effectively institute a limited deployment of the technology; this especially will be the case for self-sustaining approaches that are anticipated to persist.

Results of Phase 3 testing will form the basis for determination by regulatory and other authorities as to whether the technology should move into wider scale application as part of a national or regional program for vector and disease control. A decision to deploy the GMM method broadly for vector control will initiate **Phase 4**, which is an ongoing surveillance phase that will assess effectiveness under operational conditions, accompanied by monitoring of safety and efficacy (both entomological function and epidemiological impact) over time and under diverse situations. Long-term surveillance of

[10] For example, http://www.liebertonline.com/doi/pdfplus/10.1089/153036603322163475 or http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/PC2-4/$FILE/PC2ARTHv2-1.pdf

safety for human health will be analogous to the pharmacovigilence[11] applied in medicine, but in the case of GMM aspects of environmental safety may also be incorporated. Ongoing monitoring will be needed to ensure sustained quality and performance for disease control, and determine whether any changes are needed in management of either the GMM technology itself or other aspects of an integrated control program. In this regard, it will be important to ensure that a perceived decrease in the disease threat following implementation of GMM does not lead people living in the area to become complacent and revert to behaviors that could increase transmission pressure.

As described in subsequent sections of this *Guidance*, the transition from one Phase to the next will be subject to defined "go/no-go" decision criteria, including efficacy and safety endpoints, as well as regulatory and ethical approvals. For simplicity, the illustration in Figure 1.1 describes a unidirectional pathway. In practice, repetitions of some segment(s) of the pathway may be required in order to improve the technology and refine the procedures until the requirements for moving to the next phase are met.

Critical path for GMM development

While proof of concept for efficacy of the GMM technology is one component of the critical path, other key elements must be engaged for proof of acceptability as well as proof of deliverability and sustainability (Figure 1.2). Proof of acceptability involves risk analysis (which may be paired with cost-effectiveness analysis so as to allow comparison of risks versus benefits for alternative control measures), community and stakeholder engagement and regulatory approval. Proof of deliverability involves development of an operating model with planning for sufficient technical capacity to support wider-scale deployment, production capability at appropriate scale, financing to support deployment and subsequent monitoring, methods for field-applicable high-throughput monitoring for quality control, mitigation capability in case of adverse events, and ongoing stakeholder engagement. Sustainability will have different implications depending on whether the GMM technology is self-limiting or self-sustaining, but in either case an important aspect will include planning the response should indications of resistance to first-generation GMM be detected during Phase 4 monitoring. As is the case for drugs and insecticides, this may require support of ongoing research to develop next-generation products.

Challenges remain in identification of a viable model for the development of GMM as public health tools. Public agencies and philanthropic funders often provide the resources for Phase 1 and 2 research. However, the level of support that will be required beyond early, small-scale, Phase 3 testing may be beyond the capacity of such research funders. In the standard business model used for drugs, vaccines and insecticides (including those against malaria and dengue), industry would be expected to pick up a promising lead and provide additional financing for its development into a marketable product. However, GMM are a new technology primarily being developed for use in low to middle income countries and their potential for direct financial returns is uncertain (especially with self-sustaining versions). Small biotechnology companies with limited resources currently represent the only industry involvement in GMM. Public-private partnerships, non-profit corporations, and other models of broadly supported funding consortia may provide good precedents for GMM development.

[11] http://www.who.int/medicines/areas/quality_safety/safety_efficacy/pharmvigi/en/index.html

This guidance framework is focused primarily on considerations for proof of efficacy (testing for entomologic and epidemiologic impact) and acceptability (biosafety, ethics and engagement, and regulatory requirements), as the most immediate issues to be addressed in the critical path for GMM development.

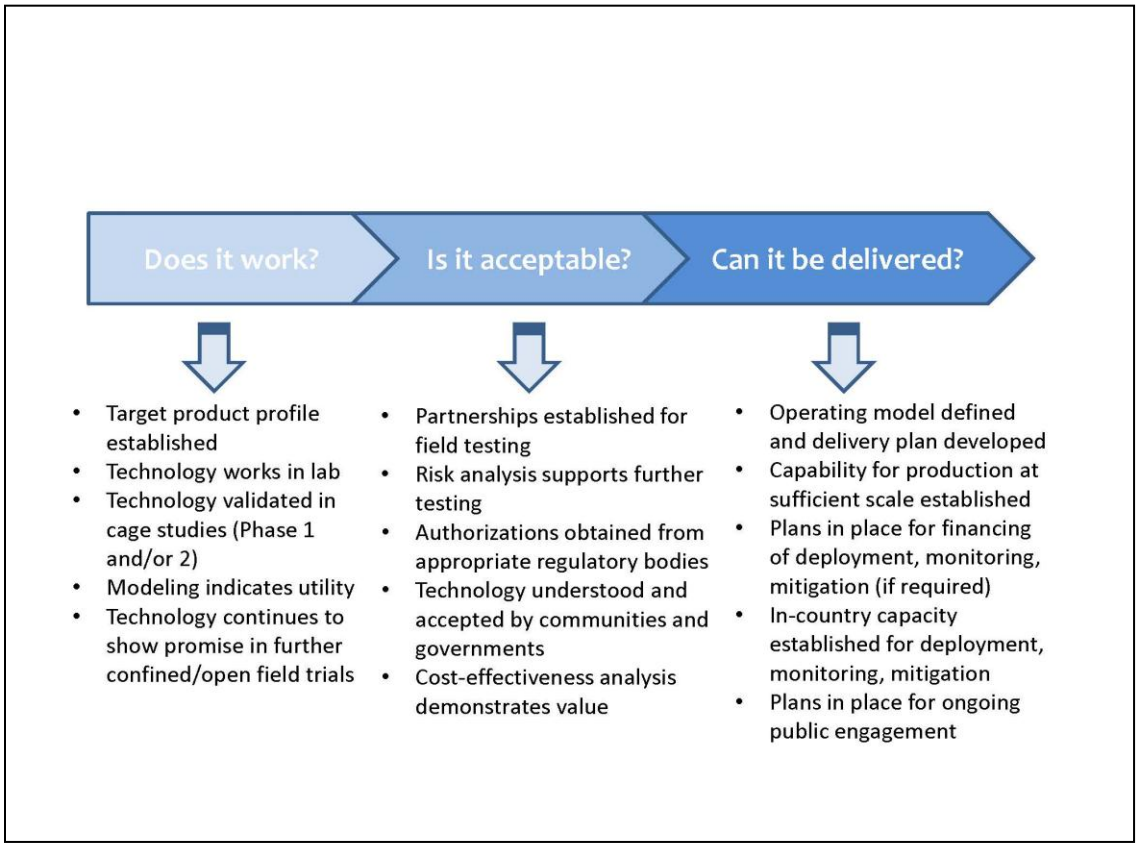

Figure 1.2 Elements of the critical path for GMM development and deployment

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2. Efficacy Evaluation

Summary: *Both entomological and epidemiological endpoints may be used to test the efficacy of genetically-modified mosquitoes (GMM) in reducing morbidity and mortality from vector-borne disease. The entomological endpoint is a reduction in the likelihood of disease transmission due to mosquito population characteristics, and will be the predominant outcome measure in Phase 1-2, and possibly early Phase 3, trials. Because this is difficult to measure directly, surrogate indicators may be chosen, and these may include vector population size, transgene frequency, ability to support pathogen replication and/or GMM fitness. The epidemiological endpoint is a measurable reduction in incidence of infection or disease in human populations. Epidemiological outcomes will be detected most easily when trials are conducted in high-transmission settings. The specifics of conducting such trials will differ for the malaria and dengue interventions that are the focus of this document. These differences include the fact that persistent endemic transmission locations are available for malaria intervention trials, and effects therefore may be observed more rapidly and unequivocally than in dengue trials, which are likely to be conducted in locations where transmission is more heterogeneous and thus less predictable. Cluster randomized trials offer a powerful design for Phase 3 for efficacy evaluation in field trials. Trial design must consider that significant seasonal and inter-annual variations can be expected. Due largely to density-dependent larval survival, non-linear relationships between entomological and epidemiological outcomes also may be anticipated. Much of the entomological monitoring required will employ methods used in any vector control program. However certain monitoring measures, such as phenotypic stability, will be unique to GMM. "Go" and "No-go" criteria for moving to the next phase of testing should be determined prior to trials. Specific entomological and epidemiological measures are recommended for each phase of testing.*

It is envisioned that GMM will be implemented in area-wide control programs. These are conducted over large areas that may include several communities and contain at a minimum the generational dispersal range of the target species. Area-wide control depends on treatment of such large regions for success, particularly in situations where effectiveness of the control measure will be influenced by the potential for reinvasion. This implementation scale stands in contrast with interventions such as repellents or nets that are effective on a household or individual level. The scale of testing and exposure of entire populations to GMM interventions have implications for how trials can be conducted. Preliminary experiments can be conducted in laboratories and outdoor cages, but testing during Phases 1-3 proceeds through increasingly larger-scale releases (Figure 1.1), ultimately to open-field releases in which the efficacy of the technology can be assessed most realistically.

While GMM technology has not yet been tested extensively in the field, experience gained from conventional mosquito control programs using methods such as indoor residual insecticide spraying, outdoor space spraying and larviciding can help predict its efficacy. Experience from sterile insect control programs on agricultural pests also will be helpful in predicting outcomes, since population suppression or preventive releases are the most immediate aims of currently-planned genetic mosquito control. Although conventional insecticidal control is usually not species-specific, its effects are similar to self-limiting GMM in that they are not permanent. This self-limiting nature provides a degree of intrinsic safety, in that implementation can be halted to reverse effects.

This chapter focuses on three key issues of efficacy evaluation: 1) the definition of entomological and epidemiological efficacy end-points of GMM; 2) methodology issues and considerations related to empirical measurement of efficacy; and 3) empirical measures of efficacy in four different development Phases. These guidelines relate to malaria and dengue vectors, as these are the applications whose development currently is most advanced and whose biology represents many other vector-borne disease systems. Other disease vectors also may become targets of GMM control, but details for determining the efficacy of these will not be discussed specifically.

Feasible applications of GMM that will not be addressed in this chapter include those in which mosquito control agencies might want to use GMM against the threat of disease or introduction of a vector. For example, such a preventative release is used in California and Florida, USA, where it is accomplished by conventional SIT programs against med fly[12]. Powerful population suppression by GMM strategies could find a market against pest mosquitoes in mosquito control programs, even where disease transmission is not a major consideration. In such cases, the entomological outcome of the frequency and scale of target-species outbreaks would be sufficient to demonstrate efficacy. Similarly, the release of GMM containing drive mechanisms to spread refractoriness in a population might be used to preclude the onset of transmission. Arguably, if such a protection were inexpensive, stable and acceptable, it might be implemented with minimal proof of efficacy against disease.

Efficacy end points of GMM

The efficacy measurements of GMM can be defined by entomological and epidemiological outcomes. These differ according to the disease, the vector species and the epidemiological circumstances. Endemic disease situations are common for malaria and the effects of interventions during trials conducted in such locations may be determined more rapidly than for dengue, which is often spatially and temporally heterogeneous. These differences, as well as the occurrence of multiple vectors in one place (particularly for malaria) determine the measures of efficacy that are appropriate and feasible. Researchers planning trials must consider not only what is ideal, but whether field sites are available for determining specific epidemiologic outcomes by the most powerful protocols.

The epidemiological end point is a reduction in infection or clinical disease incidence

In trials designed to prove epidemiologic impact, reductions may be measured by various means including infection incidence, clinical disease incidence or prevalence of infection in at-risk populations. It generally will be the case that trials designed to detect a decrease in incidence of infection will be able to achieve a statistically meaningful result with a smaller cohort size than trials that measure decreased incidence of disease, since only a subset of those infected may develop overt disease. Reduced infection incidence is generally expected to result in decreased mortality and morbidity, though this will not always occur: For example, during resurgence of disease in a naïve human population, unusually high rates of morbidity and mortality may occur. Multi-year data collection may be required to demonstrate positive effects where disease is epidemic, highly variable year-to-year or of low prevalence.

The entomological end point is a reduction in the likelihood of disease transmission due to mosquito population characteristics.

The entomological measure of transmission (also called force or intensity) due to mosquito population characteristics is the Entomological Inoculation Rate (EIR). EIR describes the degree of

[12] http://www.cdfa.ca.gov/phpps/pdep/prpinfo/

infection risk that a human population is exposed to for a particular disease as determined by assessing the vector mosquito population. It consists of the frequency of infectious mosquitoes feeding upon a person within some unit of time e.g. bites per day or year. EIR is influenced by several factors that are specific to the geographic area, including climate, bionomics of local vectors and socio-economic factors. Only transmission characteristics determined by the vector will be affected by GMM. Accurate measures of EIR are most easily made when the prevalence of a pathogen is high – hyperendemic disease transmission scenarios – and most difficult when prevalence is low or in epidemic situations. It should also be anticipated that the level of disease transmission may change during trials for reasons unrelated to the trial itself, unusual weather being the most common influence. Researchers designing the trial should prepare for such eventualities by proposing variations of the protocols during the planning phase and considering the need for adaptive management during the trial (assuming this is acceptable to regulatory authorities). The EIR varies widely in time and space in regions of epidemic transmission, and its direct determination will seldom be feasible. In practice, its measurement requires analysis of field-collected mosquitoes - often in large numbers and over long periods of time - for the presence of infective pathogens, so it can be determined only in the presence of at-risk human populations.

While a measured reduction in the EIR is the most desirable of entomological outcomes, demonstrating this will be difficult or impossible during confined Phase 2 and many Phase 3 trials. This difficulty will be particularly great when there is the potential for substantial heterogeneity in transmission, as is common for dengue. Furthermore, it is anticipated that ideal testing locations for GMM will be chosen in part for their confinement characteristics (ecological or physical islands) and the number of vector species present. These specifications will limit further the range of transmission scenarios and specific field sites that are available.

For these reasons, it is necessary during Phases 1 and 2 to infer reductions in EIR by surrogate vector indicators that contribute to the EIR. These may include daily survival, changes in absolute density, altered propensity for feeding on humans, frequency of anti-pathogen effector genes and intrinsic competence for developing infection. These indicators can be measured directly or calculated from measurable data, e.g. the realized frequency of an anti-pathogen effector phenotype in a population or the rate of spread of a transgene. The specific characteristics of GMM also must be considered in determining which indicators will be most useful to measure. For example, the frequency of GMM that suppress populations in part by providing larval competition before the lethal effect occurs will have different effects on adult abundance from GMM that produce no progeny. Therefore, monitoring larval transgene frequency and egg number have predictive value but hatching rate is less diagnostic.

Beginning in Phase 2, feeding of mosquitoes using blood from infected persons may provide a useful indicator if the GMM are expected to have reduced intrinsic competence to support pathogen replication. Such tractable measures then can be used to model the potential effect on EIR under various transmission conditions. Carefully measuring these during Phase 1 and 2 and integrating the outcomes into transmission models is an essential part of predicting efficacy. Use of surrogate efficacy measures may be necessary even during Phase 3, and will help to determine the need to move to large trials for epidemiologic endpoints.

Empirical measures of GMM efficacy

Trials must be designed to allow measurable reductions in incidence of infection

The measurable epidemiological outcomes, reduction in incidence of infection or disease in human populations, are few relative to the various GMM technologies that may be undertaken to accomplish them. Therefore, considerations for measuring these outcomes are discussed before proceeding to the variety of entomological measures and considerations of efficacy that will apply to population suppression and replacement strategies. Differences in detection and transmission dynamics between malaria and dengue will be discussed separately after commonalities are described. The end points for either disease in the context of GMM applications are similar, but the means by which these can be measured differ.

A statistically sound epidemiological trial design must be selected

The Cluster Randomized Trial (CRT, [Hayes *et al.*, 2000]), in which groups of people are evaluated (as opposed to individuals), is anticipated to be the most powerful design for detecting efficacy of GMM applications in Phase 3 trials when an epidemiological outcome will be measured. Longitudinal studies with enrolled cohorts are recommended to determine infection incidence. Passive case detection may be implemented for each cluster to determine the effect on clinical disease incidence; however active case detection is preferred whenever resources are available. The most accepted malaria [13] and dengue fever[14] case definition should be used. Good Clinical Practice[15] (GCP) should be followed.

Careful site selection increases the likelihood of detecting significant results

Detecting statistically-significant reductions in epidemiological measurements would require a large number of clusters that may not be feasible in sites with low infection or clinical disease incidence. Therefore, particularly for malaria, which often occurs at high EIR, trials in endemic areas are recommended. It is considered likely that a GMM intervention that is effective in an endemic area also will be effective in lower transmission conditions while the reverse cannot be assured. Phase 2 and 3 trials should aim to detect an effect in one transmission season. Because dengue and malaria transmission vary from year to year, multi-year trials may be necessary to ensure that both low- and high-transmission years are included in the study.

Mosquitoes disperse locally, but long distance movement by malaria and dengue vectors unaided by human activities or large weather events has not been observed (Service, 1997). However, movement of mosquitoes can confound interpretation of releases and prevent a positive trial outcome both by immigration of wild mosquitoes and emigration of GMMs. When wild mosquitoes move from untreated areas into the treatment areas, the degree of sexual sterility or increase in transgene frequency will be reduced relative to that that would be achieved in closed populations. In contrast, a self-sustaining drive mechanism with intergenerational effects may spread a gene well beyond the site of introduction and contamination of control areas must be prevented or accommodated in the trial design. Therefore, effects will be demonstrated most easily when repopulation of treatment areas by untreated wild mosquitoes and dilution of the GMM is minimized by strong isolating factors. If the GMM is a rapidly self-limiting one, separation of two kilometers will likely be sufficient (Service 1997), but if a self-sustaining GMM is being tested, separation distances must be greater in proportion to the

[13] http://www.cdc.gov/ncphi/disss/nndss/casedef/malaria_current.htm
[14] http://www.cdc.gov/dengue/clinicalLab/caseDef.html
[15] http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf

expected rate of drive. Thus, the clusters for both types of technologies must be sufficiently isolated so that the GMM are confined to, and excluded from, experimental and control clusters, respectively. Physical or ecological islands, or sufficient geographic distances, may prevent results from being confounded by inadvertent cluster contamination. Measurements of dispersal (commonly determined directly by mark-release-recapture or estimated from population genetic studies) and previous studies can guide the selection of conditions that provide sufficient isolation for various GMM, and these must be confirmed prior to trials. GMM that contain genes encoding visible markers such as fluorescent proteins can be distinguished easily from wild-type mosquitoes. Large-scale gene amplification technologies (PCR) to detect a molecular marker also are feasible. Other temporary markers such as fluorescent powders also can be useful to distinguish dispersal when populations already include GMM.

On-going disease control measures must be considered

Phase 2 confined-field trials and Phase 3 open-field trials likely will use GMM as a part of an Integrated Vector Management (IVM) program. Therefore, the effect of on-going control measures on the outcomes of the GMM trials must be considered. It is neither experimentally necessary nor ethically acceptable to test GMM under conditions in which on-going vector control activities are not continued. Therefore, site evaluation should include entomologically and epidemiologically similar field sites in which the same standard of care is being applied. Likewise, it also is necessary to continue any control activities being conducted when CRTs begin and efforts made to ensure that they are applied uniformly across sites. A change in use of conventional control methods during testing (as might be the case if those living in the trial site stop practicing other avoidance measures because they perceive a diminished threat) could change the transmission dynamics on which trial design was based. Thus, there are both scientific and ethical reasons to ensure that the trial is understood to be a research effort with no guarantee of protective effect.

GMM should be compatible with conventional control measures unless those measures exploit some weakness peculiar to the GMM (Alphey *et al.*, 2010). For example, if high levels of insecticide resistance occur in wild populations and the GMM is susceptible, then continued use of the specific insecticide(s) to which the wild population is resistant will diminish or nullify GMM effects. Therefore, considerable thought should be given to the phenotypes of the wild and GMM mosquitoes, and the control measures that will be applied for CRT site selection before making final choices.

Attention also should be given to ensuring that no major differences exist in individual human behaviors between clusters or trial sites that may affect the intervention (WHO, 1997) e.g. the use of personal protection measures (including mosquito nets), domestic use of insecticides, occupation, or migration and human movement between treated and untreated communities. Information may be obtained through interviews that may be supplemented by direct observation (e.g. of antimalarials, bed nets or insecticides available in the home). For lengthy trials, consideration must be given to the potential that new control measures (e.g. vaccines) may become available, and decisions made in collaboration with public health officials about how such a situation might be handled.

Comparative efficacy between GMM and conventional vector control

GMM ultimately may be considered as a substitute for conventional vector control (e.g., ITN, IRS or environmental management) if there is evidence that GMM may be more cost-effective. Alternatively, GMM may be combined with conventional vector control if the methods are complementary and synergistic effects are anticipated. The synergistic effect of combinations of two vector control methods can be determined if one treatment area is subject to both methods and the control area utilizes only conventional vector control. To compare the efficacy of GMM and

conventional vector control, a Phase 3 trial design should include a GMM as one case and conventional vector control as the other case. However, design of such comparison trials must be considered carefully to ensure that the population in the GMM arm is not subjected to unnecessary risk in the absence of standard control methods. Such trials should be justified by adequate prior demonstration of GMM efficacy. Phase 3 entomological and epidemiological end points described above should be measured. An appropriate number of clusters should be used to allow sufficient statistical power to detect differences. Cost-effectiveness analysis of GMM, conventional vector control, or combination of the two methods, should be performed.

Special considerations for trials of dengue interventions

Since dengue transmission is highly variable, it is likely that trials must be conducted on large spatial and temporal scales, with large numbers of clusters, in order to detect an epidemiologic effect. Large reductions of normally high transmission could be easily measured. But more typically, even a GMM trial that completely eliminates transmission might need to extend over several years to provide sufficient statistical power to conclude efficacy. GMM technologies are expected to reduce the likelihood of transmission for people within the area under management, rather than specific treated individuals within it. Thus, the area should be large enough that large numbers of individuals are not being exposed routinely to unknown risk of infection when traveling outside of their respective control or treated area, which could confound interpretation of trial results. Ideally, trial planning will include methods to allow individuals becoming infected outside of the trial area to be identified so that their contribution to incidence can be discounted. The trial plan also should anticipate variation in transmission levels that may necessitate changing the scope of the trial (for example, Phillips-Howard et al. 2003).

A reduction in clinical disease incidence may be a possible measure of efficacy when dengue transmission is high. An alternative method, which is likely to be more feasible given the expected heterogeneity of transmission, will be to measure the frequency of individuals positive for dengue antibodies in blood samples (Endy *et al*., 2008). In areas where incidence is low, reduction in dengue virus-specific IgM and/or IgG antibodies obtained by sero-survey can provide an effective epidemiological end point. Performance of serologic plaque reduction and neutralization assays in a longitudinal cohort trial, accompanied with active surveillance for virus recovery on a subgroup of people with clinically-apparent infection, may allow more accurate information on dengue risk. The need to evaluate impact on the four different dengue virus serotypes must be kept in mind.

Because regional dengue transmission is usually due to a single vector species, if GMM are effective, and achieve and maintain local elimination of that vector, then it may be unnecessary to demonstrate epidemiological outcomes as a determinant of GMM efficacy. In such a case, vector elimination can be used as the efficacy measurement. However, vector abundance *reduction* does not necessarily translate directly into reduction of dengue incidence, as transmission has been observed in the presence of low apparent numbers of mosquitoes. Determination of the threshold of vector abundance reduction required to achieve significant reduction in dengue disease incidence requires epidemiological modeling and empirical studies, and such threshold vector densities may vary among geographic localities. In the case of vector population replacement by GMM, measurement of disease incidence reduction relative to untreated controls, despite being costly, should be performed to provide high confidence in the efficacy of this novel GMM strategy.

Special considerations for trials of malaria interventions

Epidemiological outcomes for malaria must confront the multiplicity of vectors and, to a lesser extent, parasites. The high levels of malaria transmission encountered in much of sub-Saharan Africa mean that measuring epidemiological outcomes is more certain than entomological outcomes in individual vector groups, but careful site selection will challenge those designing GMM trials; not only should efforts be made to find sites matched for human demographics and disease patterns, but the selected sites also must provide sufficient confinement to satisfy the requirements of risk assessment and trial design.

Several methods are available for malaria diagnosis[16]. Historically, the "gold standard" has been microscopic examination of blood smears. However, many rural clinics lack necessary microscopes and trained personnel for malaria diagnosis. Consequently, the non-microscopic- rapid diagnostic tests (RDTs) have become popular in various endemic settings. Currently, as many as 86 malaria RDTs are commercially available from 28 manufacturers[17]. Based on the specificity of the tests, some can only detect *P. falciparum*, while others also can detect non-*P. falciparum* infections. For applications under field conditions, RDTs must be stable, simple to use, easy to interpret, and sensitive to clinically-significant malaria. The commonly recommended lower detection limit for *P. falciparum* infection is ~100 parasites/µl of blood. The specific RDT for malaria diagnosis used in a trial must be carefully selected and evaluated thoroughly according to the WHO guidelines.

Most malarious areas contain one or two dominant vector species, and it may be difficult or impossible to restrict testing of GMM to sites containing only the target mosquito. If unusual single-vector sites were used for trials, the results may not be generally applicable. However, it is clearly not reasonable to determine epidemiological efficacy accurately during Phase 2 and Phase 3 by targeting a single species when it is well established that numerous other vectors of the same disease are present and are sufficiently abundant to maintain high levels of transmission.

Experiments and modeling should be conducted prior to GMM field-testing to determine in which seasons and ecological contexts the GMM has a reasonable chance of affecting epidemiological outcomes. For example, preliminary experiments or historical records may reveal the contributions of individual vector species to the overall disease transmission levels. While these are often considered additive, each species' contribution may not conform to such a simple relationship, especially when the efficiency (vectorial capacity) of one key vector species is much higher than others. Furthermore, suppression of one target species may cause niche replacement by other, closely-related vector species. Interpretation of epidemiological outcomes by GMM in multi-species sites requires caution. These issues should be anticipated as early as possible, as with choice of target species in GMM design and selection of trial sites when entering into field testing.

Entomological efficacy must be determined in the context of the anticipated use of the GMM technology

Few interventions will be implemented in isolation, thus their performance will be determined best in the presence of other anticipated control measures. Indeed, it is an accepted procedure to conduct efficacy trials for new products in the presence of the standard of care for disease control in the area. If the anticipated use of GMM is to further reduce or eliminate populations that have been suppressed by seasonal depression or conventional methods, then the efficacy of the GMM should be

[16] http://www.who.int/malaria/diagnosis_treatment/diagnosis/en/index.html
[17] http://www.wpro.who.int/sites/rdt

evaluated in that context. If the intended use of GMM is to replace the conventional control methods, the cost-effectiveness and reliability of the GMM needs to be compared with these methods. The reliability of the GMM as a component of the suite of interventions is a central consideration. Particularly for developing countries, a GMM that is highly effective under ideal circumstances will be less attractive if it performs poorly when logistic, management or ecological difficulties arise and are common. The ability to provide for the ongoing cost of an intervention should be a consideration.

The specific experimental designs to be used may vary widely by the specific mosquito, study site and country, and the progression of experiments from the laboratory to the field will require reconsideration at each stage. When possible, the validity of a specific experimental design should be assessed by the peer-review process. In non-academic circumstances where funding does not ordinarily require peer review, independent review by experts is recommended strongly.

Surrogate endpoints must be chosen for early phase testing

GMM strains are built for specific circumstances where their potential for reducing EIR has been investigated and predicted with mathematical models. These models highlight key performance characteristics that then can be measured in the laboratory to the necessary precision as a first approximation of field performance. The performance characteristics vary with the specific strategy but include population suppression, appearance of sexual sterility, mating competitiveness, spread rate and frequency of a transgene in a population, and appearance of a particular phenotype. Measurement of entomological surrogate indicators for EIR requires close supervision and dedicated well-trained staff. In the case of self-limiting population suppression, vector abundance and its effect on EIR are the most direct measures of entomological efficacy and standard methods are available to determine these (Silver, 2008; WHO, 1975).

During the course of the trials, experimental outcomes should be used to re-parameterize computer models of the intervention. These changes may require alterations of the trial or the outcomes that can be expected. Model performance also should be monitored during the trials to determine whether its predictions are validated by trial observations.

The influence of seasonal and inter-annual variations on trial design must be considered

Seasonal and inter-annual variations in climatic conditions and other intervention measures that affect vector abundance, species composition, transmission intensity and disease incidence are common. Phase 2 GMM trials that involve small-scale, ecologically-confined field releases, and Phase 3 testing that involves large-scale open field releases, should take these variations into consideration to ensure experimental success and to enable the results to be generalized.

Self-limiting population reduction GMM will require regularly- scheduled releases when seasons are favorable, and a reduction of the population size could be a fortuitous characteristic of a specific season alone, but one that might not be repeatable. Multi-year evaluations are recommended to provide a robust evaluation of both the effects of climate and co-interventions, as well as provide an idea of how the intervention effect varies as a function of annual medium-term variations.

Population replacement in which a gene drive system is involved may take several years after repeated releases to increase the frequency of refractory alleles to an effective level. In this case, mathematical modeling should be conducted to determine the necessary trial duration for evaluating efficacy. The expected rate of spread should be considered since changes may occur in complementary

interventions for unrelated reasons. These changes may affect the efficacy of the GMM and usefulness of the original experimental design for detecting significant outcomes.

Non-linear relationships between entomological and epidemiological outcomes can be expected

The simplest outcomes to measure when GMM sterile-male methods are used are reductions in female fertility. This is typically determined by a direct measure of the number of larvae produced per female. It can be performed using laboratory-reared mosquitoes or by obtaining eggs from blood-fed field-collected females. While it may seem that increases in sterility would lead to reductions in adult populations, there is seldom a direct relationship due to the dynamic nature of larval competition. Two kinds of effects are expected: (1) negative density dependence[18] (Juliano, 2007; 2009) is common and will tend to dampen the initial effects of reduced fecundity on adult population sizes. These interactions mean that different GMM self-limiting male sterility approaches will perform differently (Yakob & Bonsall, 2009). (2) Over-compensation[19] under some circumstances may cause increases in the adult population size when larval density decreases. Both of these effects occur due to competition for food in larval sites. Knowledge of the population dynamics as determined by larval abundance would be a useful predictor of the levels of releases and sexual sterility that will be necessary in order to realize particular levels of population suppression. Ecological studies prior to releases should be performed to determine the characteristics of sites and predict the usefulness of GMM interventions.

Reductions in vector abundance or increases in refractory transgenes to a high frequency should lead to a reduced EIR. In the particular case of malaria in hyperendemic areas, this desirable entomological outcome will result in reduction of disease only when EIR falls below a threshold necessary to maintain transmission, often cited as one infective bite per year (Shaukat *et al.*, 2010). In such areas a substantial reduction in transmission intensity by the GMM or combination of interventions will likely be needed to demonstrate an epidemiological impact.

Entomological monitoring unique to GMM

Most of the characteristics used to monitor GMM functionality are not unique to the technology. Methods to evaluate these characteristics have been developed and are used routinely to gather entomological data. These include determining adult abundance, host preference and/or the ability to develop and transmit parasites or virus. These and other biological characteristics should be catalogued thoroughly during GMM testing. GMM production should utilize Standard Operating Procedures (SOPs) and Good Manufacturing Practices (WHO, 1992). Reproducible life history and phenotype can only be expected if the mosquitoes are reared and maintained using standardized procedures.

Molecular properties

A thorough description assures that changes in salient features, including the transgene sequence, its insertion site and strain background, can be detected. The description of the GMM should include information about strains that contributed genetic material to it. Variations in expression of a transgene should be quantified so that significant deviations in novel environments can be detected.

[18] Population regulation in which increased population density reduces its rate of increase. In this case, adding more immature individuals to a population does not proportionally increase the number of adults.

[19] Population regulation in which reductions in some stage of the population actually increase population size e.g. by improving survival to adulthood.

Phenotypic stability

Among the few characteristics of GMM that are unlike those monitored for typical entomological surveys, phenotypic stability is paramount. This can be evaluated by answering several questions: does the mosquito exhibit the design characteristics in both laboratory studies and field simulations? If the phenotype is not fully penetrant[20] but the transgene is stable, what effect on its efficacy and fitness do models predict? It will be possible to measure stability in increasingly realistic trials as the GMM moves forward through the Phases; however the process should begin in Phase 1. The genetic diversity of mosquitoes and pathogens with which the GMM interact and the environmental variation will increase and reveal novel variations in expression as advanced phases of testing become more realistic in Phase 2 and Phase 3 trials. Such measurements should continue periodically in the context of a post-implementation surveillance (Phase 4).

Loss of phenotypic expression can result even in the absence of transgene mutation and can negatively affect efficacy. Evolution of resistance to a transgene effector can occur either in the GMM strain itself (phenotypic drift or gene interaction) or in the target mosquito population following lengthy exposure. As with resistance to insecticides, this is extremely difficult to predict with high certainty from small laboratory studies, but one can measure pre-existing resistance in the target population and then monitor the phenotype in the field over time. As is evident with insecticide resistance, it is not the appearance of resistance but its frequency that mitigates the usefulness of the intervention. As described above for instability related to mutation, these effects can also be expected to become more evident during Phase 2 and Phase 3. Measuring such effects should be intensified beginning with confined Phase 2 trials while unanticipated effects can be restricted in time and space. The pathogen has the potential to develop mechanisms for evading refractoriness of GMM in the case of population replacement. Thus, during Phase 3 and Phase 4 refractoriness of GMM to pathogen should be carefully monitored.

Fitness

"Fitness" of transgenic mosquitoes has been the subject of much study and discussion (Catteruccia *et al.*, 2003; Irvin *et al.*, 2004; Moreira *et al.*, 2004; Marrelli *et al.*, 2006; Li *et al.*, 2008). Because this is a characteristic relevant to long-term population trends, it is of limited relevance to self-limiting population suppression strategies: the mosquitoes used for these approaches have reduced fitness by design. What is relevant is their ability to suppress wild populations, and for GMM intended to have a multigenerational effect (sex-ratio distortion[21] or inherited sex-specific sterility), the duration of the suppressive function. One measure of the maximal rate of effect on population suppression is the mating competitiveness value (Fried, 1971). It indicates (usually on a 0-1 scale) the relative frequency of mating of a male in question (in this case, GMM) when in competition with a reference wild-type male. However, there is no absolute value of competitiveness that precludes use of a strain since even very low-value insects (e.g. 0.2 for med fly) can effectively suppress populations if sufficient numbers are released. Nonetheless, measuring competitiveness, longevity and the duration of effect will provide indices that determine the necessary scale of releases and their efficiency and therefore are important for strain efficacy evaluation.

[20] The transgene phenotype is predictably absent in some proportion of the individuals in a population despite the transgene being present in an unmodified form in all individuals.

[21] Changing the sex ratio among progeny from the typical equal numbers of males and females to progeny consisting largely of males.

In contrast, the fitness of GMM used in population replacement and self-sustaining approaches is critical - specifically, the effect on fitness due to the transgene expressing the desired phenotype. This qualification is critical since the designed effect is not population replacement *per se*, but rather the introgression of a transgene causing a phenotypic change into an otherwise wild mosquito population. After release, recombination between the transgene and the wild genome will occur at rates determined in large part by the presence of natural inversions and homologous pairing. Therefore, fitness of repeatedly out-crossed individuals must be measured. Assuming that a transgene is in a drive system, the loss of fitness and reduction in gene frequency due to the transgene must be compared to hyper-Mendelian inheritance rates[22] due to the drive mechanism. Models can be used to predict the ranges of fitness and drive that will permit transgene spread. When a gene drive system is implemented to achieve population replacement and self-sustaining strategies, the frequency of the functional gene in mosquito populations into which the GMM has been released is the ultimate measure of this balance. While such measures can be used to refine efficacy predictions in Phase 1 testing, Phase 2 and 3 trials are necessary to develop final measures. This is because the activity of the transgene can differ depending on the genetic backgrounds in which it occurs.

A reduction in the EIR is the ultimate result in self-sustaining approaches. Even these kinds of GMM will require multiple releases over a large area and of a duration long enough to establish the transgene at a frequency in the population high enough to achieve the desired effect. When a GMM will be implemented by such multiple releases, it is of little value to conclude effectiveness based on more limited trials. For some interventions, this will necessarily increase the scale of testing required before the potential of the technology can be assessed – a requirement that should be taken into account in risk assessment.

Independent verification of results should be considered

Novel vector interventions are naturally open to critical scrutiny until their value has been demonstrated. Similarly, trials of GMM may be controversial, and even positive results may be questioned if methods and results are documented only by the research team involved. Research teams may wish to consider establishing an independent monitoring body to validate the results and their interpretation, as is routinely the case for clinical trials:

> "An independent Data and Safety Monitoring Board (DSMB), including a clinical monitor should be appointed for the trial (see Smith and Morrow, 1996). This should be an independent group that can testify that the trial protocol has been properly followed and that relevant quality control procedures have been operating for the duration of the trial. This Board should be set up before the trial begins rather than once it has started, as unfortunately is often the case (also trials in which this has not been done have often been those which have given rise to greater controversy)." (WHO, 1997)

Methods to ensure transparency and independent validation of results should be considered during the trial design, but careful thought should be given to whether a DSMB is necessary for trials that do not include epidemiological outcomes. A simpler alternative (i.e. an independent monitor or an oversight panel) may be designed for entomological outcome trials, which could be tasked with particular activities that are a subset of the full trial audit but whose scope is adequate to maintain independence

[22] An individual heterozygous for a transgene will produce progeny that are 50% transgenic in a normal non-drive system. Hyper-Mendelian inheritance is expected in drive systems, and these individuals produce > 50% transgenic progeny.

and validation. The expertise of those chosen for this role must adequately represent the knowledge to understand and analyze trial conduct and the appropriate trial outcomes such as vector ecology, behavior and population genetics and biology. The individual(s) selected for this task should not only provide the appropriate expertise, but should be free of conflict of interest regarding trial outcome.

"Go" and "No-go" criteria must be determined prior to trials

Transition from the laboratory to the field should always be planned with clearly stated performance milestones at which point the project proceeds to a more ambitious level, moves sideways to determine whether the unmet milestone is due to an artifact or experimental design issue or the trial is discontinued. For cage studies where population suppression or an increase in transgene prevalence is the goal, the researchers must establish clear ranges of performance that warrant proceeding. These performance standards should be assessed independently, for example by the oversight panel. Performance ranges can be informed by modeling the GMM performance characteristics that must be met in order to achieve the desired outcome in the anticipated ecological and geographic context at the next (initially entomological) level of testing.

The consequences of trials become greater as they move from physically confined to ecologically-confined and open-field release. Monitoring to detect untoward effects must increase accordingly. Whereas under physical confinement, unproductive effort will likely be the only "hazard" of unnecessarily extended trials, human and environmental hazards must be evaluated as GMM move to field release. These challenges are discussed in Section 3: Biosafety.

There are four definite "No-go" points: 1) Untoward and unanticipated disease transmission outcomes that can be linked to the experiments; 2) An unanticipated environmental harm results from the experiments; 3) Political or social opposition or unrest prevents the safe continuation of the trials; and 4) The phenotype of the GMM deviates significantly from the one intended. Depending on the technology, these could include: loss of sexual sterility, high rates of refractoriness failure, and deviations from expected sex ratios. In addition to a No-go trigger, remediation plans should be in place for such events (Section 3: Biosafety).

If no negative effect on human health or environmental quality is determined to result from unsuccessful trials, donor assessment of the value of proceeding and the relevant national authorities will determine whether the project should continue. It is common for sterile insect technique programs to be unsuccessful during production and release start-up, so initial failure should not be surprising. The technology developers may make a persuasive case that failures were due, for example, to mosquito production failures, unusual weather or implementation problems. In such a case, lack of efficacy does not require a "No-go" decision, but could preclude moving to the next phase until the cause of the failure is clarified and corrected.

Recommendations for efficacy measurements at different GMM testing phases

The final section of this guidance presents some recommended experimental activities for efficacy evaluation of GMM in different testing phases. It is likely that GMM will be used in the absence of other control methods in Phase 1 and large out-door cage testing of Phase 2. Conventional experimental approaches involving direct comparison between GMM cages and control cages with random treatment assignment may be used. In this case, only entomological measurements can be made, and thus the primary objective should be potential for reduction in transmission intensity as

indicated by entomological surrogates. A sufficient number of replicates should be used to detect the expected difference in the entomological outcomes between GMM and control cages.

Efficacy measurements will vary depending on the intended effects of GMM strategies and testing phases. It is expected that measurements of epidemiological outcomes will not be undertaken until entomological outcomes clearly predict a reduction in the EIR. For example, transmission intensity cannot be measured in Phase 1 testing in a small-scale laboratory setting or in larger population cages. Instead, transgene phenotype stability, population reduction and transgene spread and frequency are feasible and meaningful indicators of GMM efficacy. These must be considered within the context of the disease transmission setting in which the GMM will be tested and/or deployed.

Initially only entomological outcomes will be possible to measure: many of these 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. For example, for GMM strategies aimed only at population suppression, including self-sustaining sex-ratio distortion or sterility factors, one can measure vector population reduction or sex-ratio during Phases 1 and 2 (physical confinement) and it will be possible to add measures of transmission risk only after field releases commence. Alternatively, GMM strategies aiming at population replacement initially will be able to use only measurements such as transgene stability and frequency initially and then add EIR reduction in later phases.

The following section catalogues typical measurements and designs that should be considered to determine efficacy. Additional recommendations for conduct of Phase 1 and Phase 2 physically confined trials of GMM with a gene drive system previously have been published (Benedict *et al.*, 2008). The priority of various activities will change as experience and knowledge about performance characteristics in diverse settings is gained, but thorough strain description is an important activity to begin early in development regardless of the GMM type.

Phase 1. Laboratory population studies

Only entomological outcomes can be determined in Phase 1. Pathogen interactions can however be measured.

- Basic description of the transgene, including its sequence, insertion site and inheritance. This information will be used during Phases 2 and 3 to confirm the GMM's characteristics.
- Life-history characteristics in controlled environments
- Mating competitiveness against laboratory mosquito strains
- Frequency of GMM that express the desired characteristic and the level of expression
- When the strain is refractory, capability to host and transmit pathogen isolates
- For drive systems, rate of spread of a transgene in laboratory cage populations
- 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
- GMM release simulations in large indoor cages
- Modeling effects anticipated in wild populations

- Establishment of SOPs for GMM production and release

Phase 2. Physically and ecologically confined field trials

Physically confined, or "contained," refers to trials performed in large outdoor cages from which escape is highly unlikely due to physical barriers and special procedures. Such trials allow rapid termination and simple detection of escapees. "Ecologically confined" refers to those trials conducted in delimited areas from which escape is unlikely due to some ecological or geographically isolating factor. These include ecological or physical islands. Both types of trials may not be deemed necessary depending on the technology and setting in which they will be tested. Development teams and regulators may allow testing of self-limiting approaches in confinement without large cage tests, a decision that may be determined more by safety rather than efficacy considerations. 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[23] genetic constitution
- Frequency of GMM that express the desired characteristic and the level of expression in strains containing wild genetic background
- When the strain is refractory, capability of GMM containing local wild genetic constitution to host and transmit local pathogen isolates
- For drive systems, the rate of spread of a transgene in cage populations containing wild mosquito isolates and compared with Phase 1 predictions
- For population suppression strategies, the rate of suppression against wild mosquitoes in cage trials
- Egg hatching rates in crosses to wild mosquitoes
- GMM release simulations in large outdoor cages

Entomological activities in ecological confinement

- Establishment of "Go and No-go" criteria
- Measures of GMM dispersal
- For drive systems, the rate of spread of a transgene in wild populations and compared with predictions from Phase 1 and Phase 2 physical confinement
- 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 EIR

[23] "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.

- For population suppression strategies, refined measures of relationship between sterility and population suppression

Epidemiological activities in ecological confinement

- For refractory GMM, measures of the ability to sustain development of local pathogen isolates as an indication of potential for transmission

Phase 3 Staged open field releases

Phase 3 is likely to begin with limited releases intended to understand the delivery requirements and functionality of GMM under different circumstances, such as different ecologies, mosquito demographics and seasons. Large trials to determine epidemiologic impact should be undertaken after this information is in hand, as it will be necessary for trial design and interpretation. Randomized cluster trials are a recommended design for late Phase 3.

Entomological activities

- Direct measures of EIR when possible
- For GMM with drive systems, the rate of spread of a transgene in wild populations and comparison with Phase 1 and Phase 2 model predictions
- Measures of transgene functionality and mutation rate
- Measures of GMM dispersal and cross-species gene transfer
- For population suppression strategies, the rate of suppression of wild populations
- Model refinement based on Phase 2 entomology and epidemiology observations
- For refractory GMM, measures of native pathogen development and transmission in progeny from natural matings of the GMM to wild mosquitoes
- Methods for measuring GMM frequency and cross-species gene transfer and consideration of how long these measurements should continue (Section 3: Biosafety)

Epidemiological activities

- Disease incidence/prevalence studies during intervention trials
- Post-treatment active and/or passive disease incidence/prevalence, and consideration of how long these measurements should continue (Section 3: Biosafety)

Phase 4: Post-implementation surveillance

Like any public health intervention, GMM will require ongoing monitoring to determine whether their efficacy has diminished with time or due to unexpected effects when used in new areas. Appropriate measures of the entomological outcomes that guided deployment of the GMM must be continued after the trials cease. Depending on the type of GMM technology and the deployment strategy, multi-year follow-up may be required.

GMM that reach Phase 4 will have undergone extensive efficacy testing. Their behavior 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 implementation of insecticides used for long-lasting

insecticide treated bed nets, indoor residual spraying 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 program 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 sustained. It is likely that if the GMM is deployed over large areas that only longitudinal passive clinical case surveillance is practical. In case a loss of efficacy is noticed - analogous to the appearance of insecticide resistance with conventional control - any second generation GMM that may be created must also be tested in Phase 1 and Phase 2, and through vigorous Phase 3 testing and Phase 4 monitoring.

Entomological activities

- Direct measures of EIR under novel conditions (when possible)
- For GMM with drive systems, the rate of spread of a transgene in wild populations and comparison with model and Phase 3 predictions
- Widespread intermittent sampling of transgene functionality and mutation rate
- Wide-scale intermittent measurement of GMM dispersal and gene flow
- For population suppression strategies, sampling of the degree of suppression of wild populations
- Model refinement based on entomologic and epidemiologic observations
- For refractory GMM, 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

Capacity-building as an essential component of control measure durability

Durable efforts to conduct trials and to implement successful GMM interventions require strong intellectual understanding, cultural intimacy and logistic capabilities in locations where technologies are being implemented. Given the breadth of activities that have been described above, these require personnel and laboratories prepared to perform regulatory, medical, epidemiological, social and entomological activities. Further sub-specializations will be required: medical entomology, molecular biology, statistics and diagnostic analysis to name a few. It is simply impossible that these capacities be supplied without reliance upon well-trained national personnel.

During trial design, an explicit personnel plan for the project should include the specific types of supporting expertise that will be required and the degree to which the project can and must take advantage of national capacities. When specific abilities are lacking, a strategy for training national personnel to satisfy these needs should be planned and undertaken. Sufficient lead-time for training must be part of the trial design, and a commitment to retain trained personnel in the trial will be important to ensure continuity and allow for deep understanding of and involvement in the project. These personnel will play vital roles not only in trial conduct, but in regulatory interactions and long-term monitoring activities.

For many national staff, training opportunities will be professional highlights that will elevate them to national positions of authority and responsibility. Therefore, these individuals constitute an invaluable long-term national focal-point for future potential novel interventions by their knowledge of personnel, technologies and national regulatory and political avenues. Commitment to providing assistance for training lays a foundation for future strength and independence for national research activities.

Capacity includes facilities. Even though construction of major facilities will be beyond the resources of most trials, increases in the capacities of facilities can include provision of scientific equipment, computers and software required for the trials, as well as improvements in biosecurity required to achieve risk mitigation goals. Some structures such as entomological contained trial facilities will be so specialized that support for the construction will likely come from the trial program or in combination with other studies that could capitalize on the existence of a multipurpose facility such as the “Malaria Spheres” in Kenya. These kinds of facilities can be used to perform studies on mosquito behavior, life-history and non-GMM interventions. Coordinating investment in their construction provides a long-term foundation for wider sustained trials of vector interventions and research activities.

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3. Biosafety

Summary: *Biosafety associated with the development of GMM is focused on reducing any potential adverse risks to human health and the environment that might be posed by these technologies to acceptable levels, keeping the overall risk of vector-borne disease in mind. Biosafety for GMM can be achieved through a process of risk analysis. Risk analysis is described in terms of risk concern, risk assessment, risk management and risk communication. Risk concern relates to awareness and concern about issues related to both technology and social values, and in each case needs to be supported by evidence that a concern is valid. Risk assessment and management of GMM require the development of risk frameworks in which qualitative, and where possible, quantitative evidence is used to assess the probability that an adverse event (a hazard) will occur and the consequences associated with the occurrence of that event. Risk analysis takes into account that an event may occur but it may or may not be harmful in particular circumstances. Upon evaluation, some risks may be judged as negligible. Effective risk management can make many risks acceptable. Overall biosafety risk assessment should determine (i) the potential hazards and the mechanisms of impact for GMM on wild populations of target and non-target organisms; (ii) the likelihood and magnitude of impact of the GMM on the receiving environment; (iii) the levels and consequences of uncertainty associated with the effects, and (iv) appropriate risk management measures needed to mitigate any harm or uncertainty associated with changes to target organism populations or the wider receiving environments. Risk communication ensures that there is a well-documented explanation of what risks have been identified, how they have been assessed, what the acceptable level of risk is, and how risk management may be able to achieve acceptable levels of risk with implementation.*

Risk analysis is a proactive process in which the appropriate types and levels of GMM testing can be specified. These approaches should follow a phased testing pathway for GMM and be proportionate to the level of exposure at each phase. For example, containment in early phase trials mitigates concern about long term or large scale spread and provides an opportunity to assess the likelihood and impact of hazards for which little or no empirical data exists.

Studies in Phase 1 can provide data on risks that can be addressed by observing changes in behavior and ecologically relevant characteristics of mosquito populations in small-scale laboratory experiments and cage trials. Primarily this Phase focuses on the risks associated with target species effects. In Phase 2 trials, risk assessment data are obtained under physically or ecologically confined field trials. This Phase gathers risk assessment data to reduce uncertainty regarding effects on target species and the wider environment. Staged open-field trials under Phase 3 can gather data on broader potential risks including the effects on non-target organisms and off-target consequences that may not have been obtainable in previous Phases. In Phase 4, risk assessment should include the potential for movement of GMM beyond the boundaries of a release area and the scope of post-implementation monitoring.

Risk analysis that focuses on the phenotype (rather than the individual molecular modifications) provides a robust and appropriate approach to the assessment of GMMs. To achieve biosafety a reasonable overall assessment endpoint for a risk assessment is whether GMM implementation "causes more harm" than current practice. Risk analysis for GMM should be embedded in a broader cost-benefit analysis before decisions are made on implementation for public health purposes.

Introduction

Biosafety addresses the safe use of new technologies through the management of risks to the environment and to human health posed by the application of the new technology. At the four phases

of development and implementation of GMM strategies, described earlier in this guidance, there are four sets of biosafety questions to be considered:

- Phase 1) Laboratory: What information would be useful for risk assessment prior to a confined field trial and are any worker health precautions needed (related to GMM attributes and/or disease vector use)?
- Phase 2) Confined field: How should the GMM be confined?
- Phase3) Open field: Can confinement of the GMM be relaxed and by how much?
- Phase 4) Post implementation: What monitoring should be done, how and for how long?

These questions should be answered through established risk management protocols within a risk analysis framework that establishes the risks in the context of a national policy on environmental and human health risk acceptance[24,25,26,27].

Problem formulation begins with identification of potential hazards. Perceived hazards related to the release of GMM generally fall into three groups:

- Will release of the GMM increase transmission of the target or other diseases?
- Will release of the GMM cause a significant biting nuisance?
- Will release of the GMM result in disruption to valued ecosystem components?

It is useful during the management formulation process to identify any specific hazard of concern. Moreover, some risks may result from failure of efficacy of the GMM, but it should be kept in mind that unless failure results in one of the above hazards, it is unlikely that this in itself is of concern.

Risk is the combination of the magnitude of the consequences of a hazard (an unwanted event), if it occurs, and the likelihood that the consequences occur[28]. 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. Various examples of risk analysis processes are available, including a broad international standard[29], national environmental guidelines[30], and GM risk frameworks[31,32]. Across this range of guidelines risk assessment (RA) is defined as 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. In a staged series of testing, specific hazards would be addressed at each relevant stage. RA includes identifying realistic hazards (those for which some direct or relevant evidence has been demonstrated), weighing the strength of evidence for such hazards, characterizing the risk and developing risk management (RM) strategies (through procedures, guidelines and regulation) to accept, avoid or reduce risk through an overall framework for assessing the biosafety of GMM technologies.

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[24] Convention on Biological Diversity (2012) Guidance on Risk Assessment of Living Modified Organisms http://bch.cbd.int/protocol/meetings/documents.shtml?eventid=5037

[25] EFSA (2012) Draft Guidance Document on the Environmental Risk Assessment of GM Animals http://www.efsa.europa.eu/en/consultations/call/120621.htm

[26] Office of the Gene Technology Regulator, Commonwealth of Australia (2009) Risk analysis framework. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/riskassessments-1

[27] US Environmental Protection Agency (US EPA) (1998) Guidelines for ecological risk assessment. EPA/630/R—95/002F. Washington, USA. 188pp. http://rais.ornl.gov/documents/ECOTXTBX.PDF

[28] EFSA (2012) Draft Guidance Document on the Environmental Risk Assessment of GM Animals http://www.efsa.europa.eu/en/consultations/call/120621.htm

[29] ISO. (2009) ISO 31000:2009 Risk Management – Principles and Guidelines http://www.iso.org/iso/pressrelease.htm?refid=Ref1266

[30] UK Defra (2011) Guidelines for environmental risk assessment and management – Green leaves III. http://www.defra.gov.uk/publications/2011/11/07/green-leaves-iii-pb13670/

[31] Office of the Gene Technology Regulator, Commonwealth of Australia (2009) Risk analysis framework. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/riskassessments-1

[32] US Environmental Protection Agency (US EPA) (1998) Guidelines for ecological risk assessment. EPA/630/R—95/002F. Washington, USA. 188pp. http://rais.ornl.gov/documents/ECOTXTBX.PDF

The RA and RM strategies developed for any GMM release need to address two principles - the effects of the release on a receiving (open) environment and the effects of the release on human health.

The environmental and human health impact of GMMs will depend on their intended use and should be evaluated by proportionate risk assessments, which reflect the expected likelihood and magnitude of potential hazards for which there is evidence leading to valid concern. In countries with defined environmental policies and protection goals, these policies provide the framework for determining acceptable risk levels[33,34]. Observations of significant effects or changes at the various stages of GMM trials and implementation do not in themselves demonstrate a risk. The impact of the effects must be evaluated and the acceptability of risk is a policy decision that reflects the overall impact. During the testing Phases for GMM, biosafety is the main issue related to risk, but at the operational stage (Phase 4) decisions would also consider benefits and costs (including risk management measures and any unmanaged residual risks).

The fundamental rationale for GMM is to improve human health by providing an additional tool to reduce morbidity and mortality caused by vector-borne disease. Various baseline comparators for risk assessment are used under different regulatory regimes. The risk assessment of these novel technologies may be set against the risk of no action, which includes prospects of ongoing disease, or against conventional control activities, which additionally may include exposure of humans and the environment to broad spectrum insecticides. However, it is essential that potential risks be assessed and managed to ensure that modified mosquitoes are not more detrimental to human health (by increasing the disease burden or its severity) or to wider biodiversity (by adversely altering ecosystem structure and function). A reasonable overall assessment endpoint in a risk assessment would be whether GMM implementation "causes more harm" than current practice , as has been used in Australia[35]. More specific endpoints address harm to human health and particular qualities of the environment[36], and the elaboration of these endpoints would be the basis for studies to gather data to enable a risk assessment to be conducted.

The objectives of a risk analysis of GMM should include articulation of the risks of potential harm and communication of the sources of risk, the extent of these risks and the mitigation of these risks. At each level, risks specific to the genetic modification should be distinguished clearly from those generic risks associated with the conventional release of laboratory or factory-reared insects, although the latter should not be disregarded. Risk communication will be an important component of community and public engagement (Section 4: Ethics and Engagement), in determining what hazards are considered in an assessment and what management is acceptable.

The earlier development of GM technologies, principally for plants, provides a baseline for comparison of the differences and similarities raised with environmental risk and biosafety for modified mosquitoes. Environmental risk assessments for GM plants are mandated for national regulatory agencies in many countries, for example by EFSA (2010). These regulations follow a standard procedure

[33] US Environmental Protection Agency (US EPA) (1998) Guidelines for ecological risk assessment. EPA/630/R—95/002F. Washington, USA. 188pp. http://rais.ornl.gov/documents/ECOTXTBX.PDF

[34] Office of the Gene Technology Regulator, Commonwealth of Australia (2009) Risk analysis framework. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/riskassessments-1

[35] Murphy B, Jansen C, Murray J, DeBarro P (2010) Risk Analysis on the Australian release of *Aedes aegypti* (L.) (Diptera: Culicidae) containing *Wolbachia*. CSIRO Report http://eliminatedengue.com/LinkClick.aspx?fileticket=nMtZNaIayzw%3d&tabid=3911

[36] Office of the Gene Technology Regulator, Commonwealth of Australia (2009) Risk analysis framework. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/riskassessments-1

to assess the risk of the technology to the environment (as set out in the Cartagena Protocol on Biosafety[37]), and in many cases to human health as well. Principally, this involves assessing the characteristics of the modification at the molecular, ecological and environmental scale. Appropriate scientific evidence and uncertainty is used in the construction of RA guidance. While some of the goals and specific details will differ (such as the potential benefits of GMM in alleviating disease burden and the mobility of mosquitoes), the basis of biosafety guidance for GMM can be built and adapted from existing frameworks for GM plants. Other useful precedents are provided from experience with biological control agents and GM vaccines. Each of these technologies exhibits unique features, but it is important that risk analysis frameworks are consistent wherever possible.

Risk analysis as a component of decision-making

Both quantitative and qualitative risk analyses may be considered for GMM[38]. Quantitative risk analysis attempts to assign numeric values for the probabilities of various adverse events and to the assessment of the potential loss. Qualitative risk analysis assigns categories of risks, sometimes with relative scores reflecting the range of outcomes. Quantitative frameworks allow the expression of risk as probability distributions of adverse outcomes. Definitions and uncertainties in qualitative risk analysis can be expressed in scales that allow some approximate quantification. Once risk is assessed, appropriate RM strategies can be devised and their efficacy also may be quantified in some cases. The wider environmental RA and RM guidelines used in the United Kingdom give useful guidance on how to assess the credibility or uncertainty of evidence in risk analysis[39]. Quantitative risk analysis frameworks, as that used for releases of *Wolbachia* infected mosquitoes in Australia[40], may become useful in developing appropriate guidelines for the release of transgenic mosquitoes,. Belief networks may provide a robust quantitative framework for risk analysis that incorporates subjective evidence (Murphy *et al*., 2010; Spiegelhalter *et al.*, 1993).

Risk analyses must be undertaken on a case-by-case basis to identify and manage any adverse effects to the environment and/or human health. The components of risk analysis have been described thoroughly in several venues (for example by the US EPA[41], Australia OGTR[42], UK Defra[43], CBD[44], and EFSA[45,46]). Environmental risk assessment for GMOs usually follows a five-step process:

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[37] CBD (2003) Cartagena Protocol on Biosafety. http://bch.cbd.int/protocol/

[38] Office of the Gene Technology Regulator, Commonwealth of Australia (2009) Risk analysis framework. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/riskassessments-1

[39] UK Defra (2011) Guidelines for environmental risk assessment and management – Green leaves III. http://www.defra.gov.uk/publications/2011/11/07/green-leaves-iii-pb13670/

[40] Murphy B, Jansen C, Murray J, DeBarro P (2010) Risk Analysis on the Australian release of *Aedes aegypti* (L.) (Diptera: Culicidae) containing *Wolbachia*. CSIRO Report http://eliminatedengue.com/LinkClick.aspx?fileticket=nMtZNaIayzw%3d&tabid=3911

[41] US Environmental Protection Agency (US EPA) (1998) Guidelines for ecological risk assessment. EPA/630/R—95/002F. Washington, USA. 188pp. http://rais.ornl.gov/documents/ECOTXTBX.PDF

[42] Office of the Gene Technology Regulator, Commonwealth of Australia (2009) Risk analysis framework. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/riskassessments-1

[43] UK Defra (2011) Guidelines for environmental risk assessment and management – Green leaves III. http://www.defra.gov.uk/publications/2011/11/07/green-leaves-iii-pb13670/

[44] Convention on Biological Diversity (2012) Guidance on Risk Assessment of Living Modified Organisms http://bch.cbd.int/protocol/meetings/documents.shtml?eventid=5037

[45] EFSA (2006) Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants for food and feed. *EFSA Journal* 99: 1-100. http://www.efsa.europa.eu/en/efsajournal/pub/99.htm

[46] EFSA (2012) Draft Guidance Document on the Environmental Risk Assessment of GM Animals http://www.efsa.europa.eu/en/consultations/call/120621.htm

1) *Problem formulation*, which involves identifying the characteristics of the GMM that might, on the basis of practical or theoretical evidence, cause harm to the environment and/or human health, and determining how this harm might manifest and what/who is at risk of this harm, along with an appropriate comparator for the risk. Harm may be specified in some national environmental regulation, for example, in terms of threats to particular endangered species or habitats;
2) *Hazard characterization*, determining the magnitude of the harm if it were to arise;
3) *Exposure characterization*, determining the likelihood of the hazard occurring;
4) *Risk characterization*, determining the level of risk, the product of the hazard and the exposure;
5) *Risk management*, selection of management strategies to alleviate/mitigate any identified unacceptable risks.

An important concept of risk analysis is that while an event may occur theoretically, it will not necessarily be harmful, because either it does not have a negative effect, or the effects are not specified as harmful in regulations. As a result, many risks may be judged to be negligible. For example, this will be the case when the probability that the event will occur is extremely low, or when the potential harm resulting from the event is minimal. Even when potentially harmful events are identified, the practical level of risk to which the public is exposed can be reduced to acceptable levels by effective management.

National regulatory authorities will take the results of risk analysis into account when making decisions about whether and how to allow GMM testing in their countries. National public health agencies also would consider the results of risk analysis in deciding whether to take up GMM as a component of their national disease-control programs. The evaluation of risk, in the context of implementation, should be set against the benefits of GMM to improve human health. Benefit-cost analyses provide the framework under which the appropriate (economic, health) returns of a GMM release program can be quantified. One metric for assessing the benefits of transgenic vector control might focus on analysis of the epidemiological burden versus the economic cost effectiveness of the GMM, examining questions such as whether anticipated benefits including reduced health costs significantly outweigh the economic capital and operational costs associated with the implementation of GMM control programs. In a situation where use of GMM might obviate the need for certain other control methods, calculation of the operational costs of GMM should take into account any savings gained from reduction of other costs. Such analyses should be done at a point where sufficiently reliable information about the utility of the GMM is available to allow projections of cost and benefit. Relevant evidence might be obtained within Phase 3 trials, along with further biosafety focused information.

Appropriate comparators

The choice of non-modified mosquito comparators will be essential in RA of any hazards associated with the transgenic modification. In some Phases, such as Phase 1, the ancestral laboratory line from which the transgenic mosquito line was derived is a logical comparator. A potential benefit for this as a comparator is that genetic homogeneity could be maintained allowing precise scrutiny of the molecular modification in terms of genetic and phenotypic viability and variability. A disadvantage of using ancestral laboratory lines is that the loss of fitness (due to intensive rearing in the laboratory) may lead to a less precise RA relevant to the characterization of the genetic modification compared to wild populations. Choice of alternative non-modified comparators (such as field-derived strains of the modified species) will require careful scrutiny of the genetic background together with physiological and

behavioral characteristics. Such comparators might be more appropriate for field comparisons in later stages. For example, under self-limiting approaches, mosquitoes sterilized through more conventional irradiation methods may provide an appropriate counterpart for RA. Defining clear points for comparison, for example, a phenotypic characteristic such as adult longevity, will ensure that the risk evaluation remains credible, proportionate and focused.

The comparator for GMM in field trial Phases would be the wild-type mosquito in that location, and the comparisons at this stage relate specifically to the types of mosquitoes. However, at a field implementation scale, the novel mosquito control system incorporating GMM will be compared with a conventional control system. The comparison is related to the scale and purpose at this Phase and addresses the risks arising from the integrated systems of control.

Characterization of the GMM

The parental background of the GMM should be described, including the species and strain, the geographical source, the number of generations rearing colonies have been maintained and the extent of replenishment with wild stock. The genetic modification should be described, including molecular characterization, insertion sequences and location. The stability of the transgene is an important issue in determining if the characterization of the GMM remains valid over successive generations, which may be an important objective of Phase 1 laboratory studies.

In the RA, statements on the modification undertaken, its original derivation and the effect it confers should be stated clearly. The methods used to generate the GMM lines and the sequences, genomic locations and schematic maps may be required. Information on the flanking sequences may also be required in some cases, to identify whether new open reading frames are generated from an insertion. Original sources of vectors used for the molecular transformations, the source of donor genetic material, its size and intended function should be described. Information on the actual sequences inserted (or deleted), the size and copy number of detectable inserts, and the functional organization of the genetic material is necessary core information on the transgene. Details should be provided on the developmental expression of the transgene insert (or modification through knockout deletion based on transgenic technologies) during the life-cycle of the mosquito. The RA should account thoroughly for the molecular characterization and consider the risk associated with incorporation of molecular constructs or insertion mechanisms (for example, plasmids and transposable elements) into the modified mosquito.

A further aspect of characterization is the description of the GMM application. This should include an indication of the expected release rates, duration and spatial distribution of the GMM, along with any other measures that may be taken as part of the integrated control system (for example, suppression of wild populations with insecticides before GMM release).

Hazard characterization

Breakdown of the molecular function will lead to a loss of GMM efficacy and hence potential changes in the impact on the environment and/or human health. To assess this, under both self-limiting and self-sustaining approaches, the RA should be associated primarily with the genetic modification. For example, under self-sustaining approaches molecular characterizations must show that the transgene is sufficiently effective and the molecular construct linking effector transgenes to a drive system is sufficiently robust to ensure that release of the genetically engineered mosquito results in introgression

of the genes into wild mosquito populations (James, 2005). Appropriate drive systems are crucial to ensure that a faster rate of spread of the genetic construct occurs than would be expected under standard Mendelian inheritance (Burt and Trivers, 2006). It is important to understand the essential aspects of the population genetics of the transgenic modification as some gene drive systems might be expected to cycle to and from fixation in populations. Similarly, molecular characterizations for self-limiting approaches need to consider the expression patterns of the effector gene, including whether expression is under appropriate gene control and stable within the genome. The RA for GMM should consider the stability and specificity, in relation to the intended effect, of the transgenic material at the population-level and the consequences of incomplete or partial transgene function.

Identifying the risks associated with incomplete effector gene function within individual mosquitoes will have implications for different Phases of testing, including population-level and contained field trials. Incomplete gene expression will lead to poor penetrance (the proportion of a given genotype that express the phenotype) of a transgene in a population and hence may pose a risk to the receiving environment and/or human health. Under self-limiting strategies, low penetrance at the population-level will affect efficacy, and population trials should aim to quantify any human health risk associated with this in a vector control system. Such a risk could be managed with core quality control measures (e.g. genetic markers). With self-sustaining strategies, incomplete penetrance of a transgene may not influence the outcome of long-term control but might affect the initial success/spread of the transgene. Methods must be provided to allow for discrimination of GMMs within the environment and to monitor the maintenance of transgene integrity. This will be important for assessing GMM efficacy in later phases of testing.

It is conceivable that multiple transgenes might be used to achieve the desired effects. Synergistic genetic interactions and unexpected phenotypic consequences of multiple genes pose a potential risk to the receiving environment, and thus require RA and RM strategies. It is important to consider how to approach the RA and RM of 'stacked' events (multiple transgenic modifications) to ensure the efficacy of these sorts of transgenic modification and manage the risk associated with the evolution of resistance. Characterization of stacked events should focus principally on the stability of the inserts, the expression of the events and potential synergistic or antagonistic effects arising from the combination of the transgenic modification and the phenotypic characterization of the effects through life-table, behavioral, and/or population observations/experiments. Appropriate comparators for laboratory studies might include the conventional parental strains or the equivalent wild mosquitoes, the lower-stacked event lines (provided appropriate RA/RM advice exists) and wild-type mosquitoes. Characteristics based on the phenotype (rather than the individual modifications) and interpretation from baseline available data provides a robust and appropriate alternative to the full RA on every individual molecular modification in a stacked GMM. Therefore, RA should assess the impact of GMMs in terms of phenotypes rather than individual modifications in stacked, multiple transgenic modifications.

Unintended interactions of the GMM may result in potential hazards and may therefore pose risks to the receiving environment. Potential hazards might include undesirable changes in populations of interacting organisms, physiological or behavioral differences in the GMM that affect nuisance impacts, or increased opportunities for transmission of non-target diseases. Preliminary ecological or behavioral patterns associated with modification related to such potential hazards should be assessed through longitudinal, population-level cage trials of both GMM and non-modified comparators over time scales relevant to the patterns being observed. The use of semi-artificial micro- and mesocosm systems (Lawton 1995) in trials that aim to mimic the key aspects of the receiving environment would

allow the population dynamics and population-level characteristics of the GMM to be characterized more accurately than simple laboratory population cage studies. These small-scale laboratory or caged environments attempt to provide potential for interactions within a limited range of ecological complexity, which would provide a bridge into more comprehensive physically and/or ecologically constrained field trials. Careful choice of experimental design and planning allow a range of potential ecological characterizations to be established. These might include:

- The role of density-dependence in the population dynamics. The timing of density-driven events that affect survival, development and/or fecundity can be explored using population-cage and semi-artificial micro- and mesocosm trials, appropriate statistical analysis and mathematical modeling.
- Comparison of discrete dynamics, for example seasonal factors such as rainfall, versus continuous dynamics, such as competition for host-finding, under semi-artificial conditions allows estimates of the effects of seasonal versus aseasonal effects to be discriminated.
- Exploring preliminary release numbers/schemes (for self-limiting approaches**)** or invasion potential (for self-sustaining approaches) of transgenic lines.

Alterations to the biological characteristics of the mosquito may lead to altered interactions with target mosquito populations. Examples of harmful alterattions could include insecticide resistance or human feeding cues in reared populations. To predict the effects of a particular GMM release on the target population, it is essential that appropriate phenotypic, behavioral and population level characteristics of the modified mosquito are assessed through laboratory observations, trials and experiments. Although Table 3.1 provides a set of characteristics that are likely to be important in the understanding of the impact of a GMM on the target population, the most important and relevant characteristics should be identified and assessed on a case-by-case basis. This will ensure that appropriate risk assessment criteria are established and thorough RM strategies are in place.

The interaction of the GMM with non-target organisms (NTO) could have important consequences for ecosystem function and services. An example might be if the species is an important seasonal part of a food web of predators. Understanding the exposure of non-target species to the transgene or transgene products requires careful assessment to manage and mitigate these risks. Population-level micro/mesocosm trials could evaluate specific effects of the transgene on non-targets, where these have been identified. The choice of appropriate NTO (such as predators or competitors, decomposers) is a complex decision but could allow the preliminary effects of particular high-value interspecific and trophic effects to be evaluated. The European Food Safety Authority (EFSA, 2010) opinion for the choice of NTO in the RA of GM plants suggests the choice of these non-target organisms should focus on “the range of relevant biotic and abiotic interactions (such as interactions between plants and NTOs, NTOs species assemblages) likely to occur in the receiving environment(s) taking into consideration the range of natural environmental conditions”. Similar approaches might translate to appropriate choice of NTO in small population-level studies with GMM. With appropriate controls (with/without competitors/natural enemies/decomposers) the preliminary criteria of the RA on NTO can be established. For example, laboratory studies of competitive interactions on (non-modified) *Aedes aegypti* and *Aedes albopictus* demonstrate that *A. albopictus* larvae are superior competitors for resources compared to those of *A. aegypti*, and these observations have been reiterated in the field (Juliano, 1998; Daugherty *et al.*, 2000); this has implications for the invasion, establishment and coexistence of these two different species of mosquito (Leisnham and Juliano, 2009). The available information from laboratory and field ecological studies will provide an initial baseline for assessing the ecological consequences in terms of both benefits and hazards of GMM releases on NTO.

There may be concern about potential hazards related to possible direct human health effects arising from GMM, such as nuisance biting or allergic reactions. In this regard, it is important to keep in mind that only female mosquitoes bite humans or animals. Nuisance biting most likely would be related to mosquito numbers, and should not pose a hazard with GMM applications intended to either reduce populations or replace wild populations with similar numbers of refractory mosquitoes. Increased allergenicity of GMM has been proposed as a speculative risk to humans, though no supporting information is yet available. While ingestion has been suggested as a possible route of exposure, this is likely to be quite rare and thus unlikely to pose a significant hazard. The most likely route of exposure is via biting. The saliva of all mosquitoes naturally stimulates an immunologic response in most persons and a strong allergic response in some (Peng and Simons, 2007), and there is considerable cross-sensitivity to the proteins from wild populations of mosquitoes. Therefore, determining a GMM-specific response in the context of such natural variability will be difficult. However, with GMM technologies in which female mosquitoes will be released or transgenic material may be transferred to female progeny, it is appropriate for an RA to consider whether a transgene product protein is expressed in the saliva and if so, whether this protein is significantly similar to a recognized allergen. In this case, further studies may be warranted. For example, a hazard might be identified if evidence demonstrated that the new protein that occurred in GMM saliva caused additional reactions in sensitive subjects.

The efficiency of quality control and additional essential attributes in the modification of mosquitoes such as the operational ability to derive only certain types (for example, one sex in male-only releases) of transgenic insects for release requires appropriate RA. The methods and degree of separation necessary depend on the scale of the trial or planned release and the GMM technology under consideration. Achieving the appropriate sex ratio and levels of separation require appropriate operational protocols. In laboratory trials and population cage experiments the ability to discriminate and separate appropriate strains of transgenic mosquitoes should be evaluated. RM options should focus on how necessary it is to achieve full (100%) separation in order to achieve safety and efficacy in the trial/release. Control may be achieved even when some females, which do not contribute to control in sterile male release programs, are released. For example, in the use of a conventional radiation SIT method, the local elimination of *An. albimanus* in El Salvador was achieved with the release of sterile insects of which approximately 14% were females (Lofgren *et al.*, 1974).

Baseline information on key ecological, environmental and site characteristics of confined field trials is important to ensure that trials can be adequately planned and interpreted. Selection criteria might include having knowledge of the distribution of principal vectors in the release area, mosquito larval breeding sites availability, climatic conditions, knowledge of active transmission (if any) of the target disease pathogen at the site, geographic isolation of the site so that there is a negligible chance of any impact outside the trial area, existing data on the transmission dynamics of the target disease, existing surveillance and control systems for both vectors and disease, the likelihood of obtaining regulatory, social and political approval for research on GMM in the study community and surrounding areas (Sections 4 & 5) and the ability to continue existing vector control practices. Plans for mitigating unanticipated outcomes also must be developed and in place.

Further possible hazards could arise from the potential random integration of the effector gene, low efficiency and position effects on transgene expression and the potential for insertional mutagenesis. It is likely that transgenic strains exhibiting these effects would not be considered suitable for deployment. Therefore, most of the potential hazards resulting from random integrations are expected to be eliminated during the product development process. Strategies to reduce random integration might be employed. An example of such a strategy is provided by the two-tiered approach

to the molecular modification of mosquitoes (Nimmo *et al.,* 2006; Isaacs *et al.,* 2012): the first stage involves inserting a target at a suitable chromosomal site, and the second involves recombining the effector gene into the target site.

Utility of mathematical modeling for RA

Phase 1 assessment of risk can be enhanced by coupling the experiments and/or observations with mathematical modeling. Mathematical modeling can highlight the range of parameters necessary for RA. The overall aim of mathematical modeling within the RA context is to show predictions of behavior based on properties and assumptions of transgenic modification that may be helpful in assessing likelihood of events. For example, given a set of molecular modifications, mathematical models might be used to predict whether the fitness of the GM mosquito is enhanced (or not) by the molecular modification. In a model system where GMM containing an anti-pathogen effector gene were continually fed on mice with an abnormally high level of parasites, increased fitness of the malaria-resistant mosquitoes was observed (Marelli *et al.* 2007): modeling might be used to determine the net effect of increased fitness, the expected frequency of infected mosquitoes and possible effects on transmission. The appropriate theoretical framework to undertake this RA would be a full life-history analysis combined with competition experiments. Essentially, this consists of determining both aspects of fitness associated with survival and aspects of fitness associated with fecundity and reproductive success (Stearns, 1992; Roff, 2002). This could involve laboratory studies that focus on a selected set of core parameters (Table 3.1) associated with the specific genetic modification coupling life-table experiments, experiments on small batches of modified and non-modified mosquitoes (such as split by age, sex or strain) in cohort experiments, and mathematical modeling.

Mathematical modeling of interspecific interactions could be used to reveal potential structural alteration to the ecological (biotic) effects. For example, self-limiting strategies where population suppression is the goal are expected to lead to non-uniform competitive effects, as population interaction strengths with other species will be different at high and low densities. Under self-sustaining strategies, assessing whether the heritable modification has an impact on the ecological competitive ability of the GMM and/or ecological interactions should be evaluated through small-scale semi-artificial population trials in the laboratory.

RA and RM considerations at different testing phases

While it is important to understand the molecular characterization associated with the relevant technologies, RA and RM must be focused in terms that address specifically the particular GMM application under examination and its objectives, as well as the phased testing pathway. Specific RA and RM considerations will differ between various GMM technologies and in different phases of testing. For example, the level of exposure will be less in contained trials than open releases, and with sterile GMM versus those that are self-sustaining. In each Phase, from laboratory through to field trials, the aim of specific RA and RM approaches should be to quantify or provide a qualitative rank of risks associated with the eventual deployment of GMM. Thus, this overview of biosafety should be followed up by analyses that address the detailed regulations associated with the specific GMM approach under investigation.

Transition from each phase of testing to the next should involve both a retrospective validation of the RA/RM that was put in place at the beginning of the phase and an evaluation of whether the performance characteristics that were measured warrant progressing to larger trials. In addition, any

hazards that were unforeseen before starting the previous Phase should be considered in the decision along with additional management measures.

Phase 1 – Laboratory Studies including Laboratory Population Cages

Risk assessment

This Phase of the development of a transgenic mosquito focuses primarily on aspects of the biology associated with the target species and integrates molecular, genotypic, phenotypic, behavioral and population level characteristics (Section 2). The data collected at this Phase to address identified risks will focus primarily on the genetic modification of the mosquito and its interaction with the comparator mosquitoes in the laboratory. Alterations to target populations through changes in the demographic size, structure or behavior may have a detrimental impact on the wider environment and/or human health. Experiments to determine whether these alterations lead to specific harms can already be addressed at this stage. Examples of Phase 1 studies that will characterize those aspects of the biology of the modified mosquitoes that inform the RA associated with the eventual deployment of GMMs are provided in Table 3.1.

Risk management

Phase 1 trials should be conducted in contained laboratory conditions. Because it is 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. RM measures for environmental impacts will include confinement of live mosquitoes and destruction of dead mosquitoes and waste materials (if there is evidence that these may be a hazard) as the trials proceed. RM measures for human health would include ensuring GMM colonies and feed sources are free of human pathogens, ensuring laboratory staff are not carrying mosquito-transmissible disease, and limiting unintended biting opportunities (to guard against disease transmission) by preventing and removing mosquitoes flying outside cages and by laboratory staff wearing suitable protective clothing (Benedict *et al*., 2003). RM to respond to escapes from the laboratories would include escape detection systems and standby mosquito control capacity sufficient to provide control within the dispersal range of the mosquitoes, or conducting experiments in seasons when flight and mosquito larval breeding sites are limited. Where trials are testing specifically disease transmission or infection cycles in GMM, particular care should be taken to ensure the safety of laboratory staff. All of the above also are good practices in rearing non-GM mosquitoes, particularly when they are being handled in areas where they are exotic and could establish following escape.

Results of Phase 1 testing will determine whether trials may proceed safely to Phase 2 ecologically confined trials or whether physical confinement is a necessary intermediate step to obtain additional safety information.

Phase 2 – Physically and/or Ecologically Confined Field Trials

Risk assessment

For confined field trials RA is extended to account for greater varieties of environmental and target species effects. Risk associated with these trials can be managed by limiting the spatial and/or temporal scale of the planned release activity. Physically confined (contained) and ecologically confined

field trials conducted under Phase 2 allow data to be collected that require a larger scale or more natural conditions in order to be detected. This also allows evidence to be gathered to provide an appropriate level of RM before full implementation of open field trials in Phase 3 (which are likely to be conducted in a location where the target disease is endemic). Physically confined field trials employ some type of barrier (such as screens) to prevent mosquito access to the outside environment. This type of trial introduces differences from the natural environment that may affect the performance of GMM and other organisms within the trial, so it will be important to obtain the most relevant information needed to make decisions about proceeding to the next Phase. 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, appropriate RM of residual dead material would need to be considered. Ecologically confined field trials may take place in locations that do not favor the long-term survival of the GMM, or in ecologically isolated locations (such as an area surrounded by water, deserts or mountains). Combinations of physically and ecologically confined trials are possible.

Consideration should be given to whether the release of GMMs poses a risk through the persistence of functional genetic material within the GMM species and whether the transfer of the genetic material can occur between species. The stable transfer of genetic material from one organism to another without reproduction is called horizontal gene transfer (HGT). The risk posed by HGT from GM organisms is generally believed to be negligible (reviewed by Keese, 2008). No evidence of HGT from transgenic plants to microorganisms has been detected in the field over decades and millions of acres (Keese 2008), and therefore occurrence of HGT from GMMs may be expected to be even rarer. Considerations relevant to RA for transgenic organisms, including GMM, are whether the transgenes contain components that could plausibly confer a selective advantage to microorganisms with which the GMM will interact, and whether acquisition of this trait would be harmful. RA would need to consider this on the basis of the known function of the transgene and whether that function is preserved in microorganisms.

Identification of clear endpoints to the field evaluation will require basic ecological, entomological and epidemiological information. Ecological processes such as density dependence and age structure affect the design of measures to mitigate risk to the wider environment, biodiversity and human health. This assessment should be considered in Phase 2. Density dependence is a process that leads to increased mortality or decreased fecundity as density increases. It is an important ecological process in the dynamics of most populations and evaluating its timing and effect in wild-type versus transgenic mosquito populations is of potential importance to the RA of modified mosquitoes (Yakob and Bonsall, 2009). The timing of important density-dependent processes with respect to the expression of the effector gene has substantive implications for the impact of some proposed genetic control suppression strategies. Under both self-limiting and self-sustaining approaches, timing the expression of the effector gene after the stage at which density-dependent effects are greatest (such as the larval stage of *Aedes aegypti* (Phuc *et al.*, 2007; Legros *et al.*, 2009) can lead to more effective suppression. Phase 2 trials should be structured to provide appropriate information on the ecological processes critical to the evaluation, efficacy and success of the GMM. Age structure can affect density dependence where different stages and ages within stages do not compete with each other.

Risk management

The level of confinement required in Phase 2 will be determined by RA and RM. For some GMM technologies, it may be decided that physical confinement is not a necessary step in the testing pathway and that conditions of genetic or ecological confinement allow for sufficient risk reduction. Physical confinement may be less important in cases where Phase 1 results have demonstrated there is limited potential for dispersal, for example for trials where the GMM do not mature to adults, or where the modification is not deleterious (for example, markers in laboratory strains with naturally low fitness in the wild). Previous evidence from laboratory or confined trials may demonstrate that protocols to discriminate the sex of the released mosquitoes, and their phenotypic properties, are sufficient to ensure safety in a confined trial.

RM should include monitoring of GMM populations within the trial area to ensure that the technology has the intended effect on the target population. A mechanism for practical and reliable discrimination of GMM and wild mosquitoes is essential (for example, through the use of fluorescent dyes or dusts and/or phenotypic or genetic markers). Where release of male-only GMM is part of the system, reliable sex-selection prior to release will be necessary to ensure an acceptable sex ratio is achieved. Periodic sampling of the GMM population in the trial should be undertaken to determine the stability of the transgene and any recognizable evolutionary change in the genetics of the population that may affect the impact of the technology. Key interactions with other species in the trial, which might indicate wider environmental impacts, should also be monitored to identify and characterize any unexpected harmful effects; identification of a few representative “sentinel” species will be useful in this regard. There should be sufficient monitoring for early detection of any GMM that escape confinement. Standby control capacity should be maintained that is sufficient to control any escaped mosquitoes within their potential dispersal range outside confinement. Measures may be taken to limit establishment within potential dispersal zones, such as controlling wild mosquitoes and limiting available larval breeding sites, where this is practical. Standby control measures should take into account any behavioral attributes of GMM that may differ from wild mosquitoes. Monitoring and control capacity should continue after the trial is completed for a period sufficient to ensure that there is no unintended persistence of the GMM or manifestation of unintended effects.

Physically confined field trials should give particular attention to cage designs and local environmental conditions at the chosen field site. Aspects of local geological, ecological and regulatory criteria will underpin physically confined field cage design and trial implementation (Facchinelli *et al.*, 2011; Ritchie *et al.*, 2011). Understanding the risk associated with a breach of physical/ecological confinement also requires appropriate consideration. A breach of physical confinement may lead to the loss of transgenic mosquitoes or loss of genetic material into the wider receiving environment. Breaches of physical confinement might be classified usefully in terms of the potential magnitude and type (Benedict *et al.*, 2008). Breaches might be caused through natural disasters, structural failures, human error/accidents or deliberate actions. The RA should take into account cage designs, experimental planning, emergency preparation and training and site security. Documenting the hazard/differences associated with the escape of self-limiting or self-sustaining transgenic lines through breaches will be an essential aspect to RM, including the containment requirements for cage design. It is anticipated that the risk will be lower with self-limiting GMM due to their lack of potential for persistence in the environment. Further simple RM measures including the appropriate design for the cages accounting for the local ecological and/or environmental/geological conditions of the physically confined trial, restricted access and clear and well-managed SOPs and ethical/cultural considerations all could be used to mitigate hazards associated with contained trials.

While protocols based on standard operating procedures (SOPs) would be necessary at the latter part of Phase 1 laboratory population trials, such operational procedures become even more important as testing through the tiered Phases moves to confined and open field releases. SOPs are a written plan describing procedures to be carried out in the field trial evaluation of GMM. For example, SOPs would document how transgenic material should be moved from the laboratory to the field prior to release, the protocols for ensuring site security and cage suitability (Benedict *et al*., 2008), criteria for release strategies, the surveillance during the trial and the post-trial removal of material and cages. SOPs should describe the lines of responsibility and the RM strategies and options for the trial.

Phase 3 – Staged Open Field Releases

Risk assessment

Open testing in Phase 3 will introduce opportunities to gather data on potential hazards in the risk analysis (Table 3.2) where these data can only be acquired under more natural conditions. It also provides an opportunity to evaluate the performance of GMM integrated within complementary conventional control actions. RA 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 (see Table 3.1). A failure to decrease vectorial capacity under self-sustaining approaches may result from a decoupling of the effector gene from the drive system. Vectorial capacity under self-limiting approaches also is associated with the quality control of transgenic releases. Incomplete penetrance of the modification may influence both vector capacity and potential disease burden.

The RA associated with 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. The size of the open-field release site also will dictate the site characteristics to be considered within the RA. Site-selection within an RA could make use of the substantial advances in technology and knowledge for geographic surveys (e.g. global-positioning systems, geographic information systems and high resolution satellite images) and predictive models of habitat suitability. These methodological advances allow thorough analysis of temporally- and spatially-referenced data relevant to both mosquito ecology (Thomson and Connor, 2000; Malcolm *et al.*, 2009) and disease burden (Gething *et al.*, 2010).

Choice of appropriate site size and layout (randomized block, Latin square, Cox and Reid, 2000) 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 (Section 2). 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 and 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 endpoints 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 (Section 4: Ethics and Engagement) should be incorporated into the RA.

The spatial scale of a proposed field trial may have environmental consequences through NTO effects outside the planned boundaries of the trial site. Risks associated with potential transgenic releases should consider the spatial pattern and the scale of the entomological/ecological risk (Getis *et al.*, 2003). The effects of modified mosquitoes may extend to neighboring areas if migration between populations can occur (Yakob *et al.*, 2008). Determining the appropriate scale for a release strategy and the implications for adjacent non-target regions requires an appreciation of the relationship between ecological processes such as the timing of density dependence, demographic processes (Table 3.1) and spatial aspects. This can only be evaluated realistically under field trials. 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.

Understanding endpoints and intended consequences of GMMs necessitates understanding the relevant aspects of mosquito biology and ecology. Basic ecological knowledge of mosquito vectors in potential receiving environments must be available to evaluate the benefits of transgenic mosquito releases and obtaining the necessary information should be part of the overall research plan. For example, while population genetic studies on mosquitoes are common (Toure *et al.*, 1994) there have been practically no ecological studies of the effects of seasonality in West Africa on *An. gambiae* in relation to the forms that are present and how they are distributed in space: basic information such as whether *An. gambiae* is resident in or repopulates disease-endemic areas remains unclear. The ecological difference between intrinsic population growth and immigration is substantial and requires assessment in order to validate risk estimates, define RM and determine appropriate endpoints. While extensive information on direct and indirect interactions through purposely-designed experiments would be desirable in any ecological field study (Bender *et al.*, 1984), key information for the RA of undertaking transgenic releases under open field conditions should be proportionate and focused, requiring the development of sampling programs (Silver, 2008). The impacts on human health and the wider receiving environment cannot be evaluated appropriately without this assessment.

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.

Risk management

RM in Phase 3 will be similar to Phase 2 above but will need to be extended in scale to account for the lack of confinement. An additional risk management measure would be to stop GMM releases in the event monitoring detects an otherwise unmanageable and unacceptable hazard has developed. In such a case, a more extensive and intensive conventional control capacity may be required to eliminate any residual population of GMM after release and dispersal.

There should be a procedure to monitor any degradation of efficacy in the GMM control system that may indicate 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.

Phase 4 – Post Implementation

Risk assessment

During implementation it is important to validate the cumulative RA from earlier phases - were hazards identified fully, were risks characterized accurately and were relevant management measures effective? Throughout the release program there should be high quality control standards for GMM characteristics (Tables 3.1; and 3.2) and procedures (for example, in rearing mosquitoes for release programs, determining sex ratios for release, etc) to ensure that processes remain relevant to the RA assumptions.

The release of transgenic mosquitoes is expected to have effects on target organisms through either the suppression or replacement of local mosquito populations. Failure of intended effects may pose a risk, particularly to human health if the GMM vector control system fails after a release program is well advanced. The potential for evolution and adaptive processes must be considered within the RA. This might include assessing local mosquito populations for the evolution of resistance to the transgene function, the evolution of the disease pathogen to resist transgene function or changes in host range of targeted mosquito species. While a small number of selectively advantageous genes released into an environment might not be expected to persist due to chance (Fisher, 1922; Kimura, 1962), RA should consider whether the mass-release required by self-limiting GMM approaches might introduce a selection pressure into an environment that could drive the evolution of novel biochemical or behavioral resistance. Mutations that confer resistance to insecticides are well-known, and it has been demonstrated that resistance-favoring mutations can be present in populations before the start of a control intervention program (ffrench-Constant, 2007). RM strategies should emphasize predicting the likely manifestation of any potential resistance (Alphey *et al.*, 2011) and a plan should be developed before carrying out Phase 4 field releases to monitor its frequency. The RM requires appropriate remediation strategies to be in place to mitigate the evolution of resistance. Implementation programs should plan for the potential of adaptive processes in the target population, and describe the lines of responsibility for managing this risk. Quality control in rearing facilities should continually check for any signs of failure of mechanisms integral to the efficacy of the GMM or factors that could make control more difficult. The RA process also should identify characteristics of the GMM that might change as a result of mass production that could impair the effect of the GMM including selection for altered development rates, size and marker expression. A plan should be in place to monitor for such changes.

As noted above, the GMM releases could lead to altered ecosystem functions through interactions with NTO, such as the role of mosquito larvae as food for predators. It has been argued, however, that since many mosquito vectors are invasive species in the areas where they transmit disease, their removal would return the environment to a more natural state. Under releases of GMM aimed at population suppression, alterations (reduction) in target population sizes are expected and hence potential alterations in species interaction strengths would be anticipated. In contrast, population sizes might not necessarily be altered under population replacement strategies although the transgenic modification might affect mosquito behavior or phenotype.

Although the possible effects of GMM on NTO theoretically may be extremely broad, RA and RM need to be science-based, proportionate and directed to specific hazards. In particular, the effects on

the phenotypic, behavioral and population level characteristics of the modified mosquito (for example, the parameters described in Table 3.1) on the target population should be reassessed within the scope of risks associated with full public-health implementations. Extending the assessment of the effects of the transgenic mosquito on NTO should be considered under this Phase of testing.

Risk Management

The appropriate regulatory structures, mechanisms and methods need to be in place as an integral part of the RA to ensure that clear lines of responsibility are delineated on post-implementation surveillance. Post-implementation monitoring should focus on the appropriate effects and variables (based on observations from prior testing), the time-course over which surveillance should occur, the geographic limits to surveillance and the methods by which to measure the effects. RM strategies should take into consideration whether and when it will become impractical to maintain active surveillance programs as the GMM become ubiquitous under self-sustaining approaches. Evaluation of GMM post-implementation 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). The RA should establish and delimit appropriate time intervals when the impact and continued safety of the GMM technologies should be reviewed. The post-implementation surveillance method should also be reviewed at appropriate intervals as population levels change.

Post implementation monitoring should draw on evidence from earlier Phases to determine the need for and design of monitoring to observe the key impacts identified by the CBD[47]:

- Effects on biological diversity
- Vertical gene transfer
- Horizontal gene transfer
- Persistence of the transgene in the ecosystem
- Evolutionary responses (especially in target mosquito vectors or pathogens)
- Unintentional transboundary movement

However, there should be a rationale in each of these cases by which monitoring is focused on valid concerns demonstrated by evidence of mechanisms and time scales that could lead to any of these effects, and particularly to events that reasonably could be expected to lead to harm. General surveillance approaches are unlikely to be effective or informative in determining impacts. By Phase 4, necessary monitoring methods will need to be easily scaled up and be applicable in the field.

A plan should be developed for case-specific post-implementation surveillance of GMM and any key species for which there is evidence of harmful interactions in order to assess the impact, risks and benefits once a GMM-based control program is underway. Key species may include those in the main food web interactions and any endangered species listed in national regulations. Post-implementation surveillance may be considered after the release of any transgenic or non-native organism, in order to address remaining uncertainties identified in the RA or to confirm that conclusions of the earlier RA are accurate once large-scale and long-term open release has taken place. In the case of GMM, the public health implications impose an additional obligation to ensure that the transgenic technology remains efficacious and poses no additional risks, so health monitoring of human populations in the release area

[47] CBD (2012) Guidance on risk assessment of living modified organisms. Montreal, Canada. 44pp. http://bch.cbd.int/protocol/meetings/documents.shtml?eventid=5037

should be carried out to ensure the expected levels of efficacy have been achieved. Otherwise additional conventional control may be required to reach acceptable levels of vector control.

Several potential risks with regard to human health should be considered during Phase 4. The release of transgenic mosquitoes may lead to a concern that existing control measures may be reduced, either as people become more lax about personal and household mosquito control efforts or as governments look for cost savings. The implications of a reduction in conventional vector control to mosquito population dynamics, human health and to the wider receiving environment require appropriate RA and RM. It might be that part of the RM requires certain conventional practices to continue and it may be necessary to integrate the GMM technology with these conventional control strategies.

The possibility of resurgence of disease when immunologically naïve human populations are exposed to disease after a prolonged period of low incidence is a concern that should be assessed in post-implementation monitoring. However, this is not unique to GMMs. For example, concerns were initially raised about the possibility that insecticide-treated bed nets (ITN) might increase mortality in older children through delayed acquisition of immunity to malaria. Empirical evidence from a community-randomized controlled ITN trial in malaria holoendemic western Kenya found no evidence of compromises in human immunity to blood-stage antigens in young children after two years of ITN use (Kariuki *et al*. 2003) and no evidence for increased all-cause mortality in older children six years after ITNs were provided to children (Lindblade *et al*. 2004). However, recent observations of increased susceptibility in older children and adults following long-term use of insecticide-treated bed nets have once again raised this question (Trape *et al*., 2011).

An appropriate disease surveillance program should be part of the post-surveillance monitoring scheme. It is anticipated that this could be provided in the context of ongoing national disease control programs. RM should consider whether additional case-specific surveillance methods should be put in place to monitor transgene activity within the GMM.

The release of GMM provides different, but not entirely novel, issues to those for GM plants. Arguably, the most important biological difference is the possibility for autonomous dispersal. However, appropriate biosafety assessment (Table 3.1) will provide the fundamental information for appropriate RM options. Precedents dealing with biological control and conservation of biodiversity provide additional relevant insights into how the potential for transboundary movement may be managed (Section 5: Regulatory Frameworks). Further, there are analogies with biosafety management associated with the release and use of vaccines based on GM viruses or bacteria, where individuals are inoculated with a vaccine and disperse into the wider receiving environment. Establishing the broader environmental risks of GM vaccine shedding rates is of particular relevance. The equivalent for GMM would be the assessment of dispersal and replication rates (Table 3.1) in the wider environment following an open release (Table 3.2).

The mass rearing and release of transgenic mosquitoes may have consequences (and associated risks) related to cross-border movements and spread[48] . RA of open release and post-release surveillance of GMM must consider the likelihood and consequences of spread of mosquitoes across

[48] North American Plant Protection Organization (NAPPO) (2007) RSPM 27 Guidelines for importation and confined field release of transgenic arthropods in NAPPO member countries. http://www.nappo.org/en/?sv=&category=Standards+Decisions&title=authors+27

international borders. This could have ecological consequences, but since most management activities would be national responsibilities it would be important to consider how neighboring national authorities would plan and carry out risk management actions, including appropriate surveillance, that might be needed. The movement of transgenic material across national/international borders is governed by well-established RA procedures (under the Cartagena Protocol on Biodiversity). Parties bound by the Cartagena Protocol (and its instruments) are expected to carry out the movement of transgenic material (to both Parties and Non-Parties to the Protocol) in accordance with the objectives of the Protocol (Section 5), and other regional agreements, such as NAPPO RSPM 27.

Consider the need for independent safety review

The establishment of independent safety review groups or the formulation of GMM biosafety regulations for consideration by existing review groups (local bodies such as IBC – Institutional Biosafety Committees[49], national advisory bodies such as ACRE – Advisory Committee on Release to the Environment[50] , and regional or supranational agencies such as EFSA – European Food Safety Authority[51],) is recommended. Such groups can provide oversight of the RA and RM within each Phase of testing and provide independent scientific advice on the risks of GMM to human health and the environment.

Biosafety Capacity

The successful implementation of GMM interventions requires transparent, focused, proportionate and credible biosafety assessments. National safety review groups, capable of providing appropriate independent guidance and overseeing all facets of testing and implementation, will be important for biosafety assessments of GMMs. National-level biosafety boards should draw on available expertise across a wide range of scientific, environmental and economic disciplines (for example, as provided in the CTNBio in Brazil[52] or CIBIOGEM[53] in Mexico), to assess the risks and benefits of GMM technologies. Stakeholder groups and individual communities provide the key to the ethical dimension and should likewise form a consistent and strong voice within both biosafety and benefit-cost analyses associated with the testing and implementation of GMMs.

The decision making bodies approving biosafety testing should have capacity to formulate the risk problem, to define appropriate endpoints for risk, to interpret the character of the component sources of risks, to interpret the quantification of risk components and understand the efficacy and uncertainty related to proposed risk management measures. Where this capacity is not available at a national level, efforts should be made to obtain independent international expertise, and to strengthen the necessary national expertise in the longer term.

[49] http://oba.od.nih.gov/rdna_ibc/ibc.html
[50] http://www.defra.gov.uk/acre/
[51] http://www.efsa.europa.eu/
[52] http://www.planalto.gov.br/ccivil_03/_Ato2004-2006/2005/Lei/L11105.htm
[53] http://www.cibiogem.gob.mx/Acerca/Paginas/default.aspx

Conclusions

The assessment of the safety of GMMs for human health and the environment should follow a phased approach moving from laboratory and cage experiments through to open field releases. RA and RM at each stage should provide sufficient information to determine whether a credible decision can be made to allow trials to move on to the next stage. This ensures a workable and defined protocol to follow in the development of appropriate decisions for each further testing stage.

It is obvious that not all the hazards described above will be universally relevant to all types of GMM. It is important to emphasize that RAs should proceed on a case-by-case basis and be proportionate to the particular phase of testing. Defining the potential extent of harm that could be caused to the environment or human health by GMM, identifying the risk level (hazard by exposure) and developing risk mitigation plans provide the framework in which to undertake the RA. RA of novel GMM technologies should be set against the risk of a relevant alternative comparator. The overall risk assessment endpoint should be whether GMM release "causes more harm" than current practice. Comprehensive evaluation of GMM implementation, following safety focused trials, should be considered in a broader benefit-cost analysis and the RA and RM plans form only one component of this broader analysis.

Table 3.1: Example parameters for laboratory studies on the risk assessment of transgenic mosquitoes. The risk assessment should focus on the hazard (the harm that might be produced by 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.

Parameter	RA Method	Exposure Assessment
Female Fecundity	Cohort experiment; Life Table analysis	Is it limited by population density and/or individual physiology?
Oviposition Rate	Cohort experiment	Is it limited by population density and/or individual physiology?
Egg Development Rate	Cohort experiment; Life Table analysis	Is there a significance difference?
Egg Survival Rate	Cohort experiment; Life Table analysis; Population level modeling	Is it density-dependent? What is the type of density-dependence? Is it under/overcompensatory? Does it differ?
Larval Development Rate	Cohort experiment; Life Table analysis	Is there a significance difference?
Larval Survival	Cohort experiment; Population level modeling	Is it density-dependent? What is the type of density-dependence? Is it under/overcompensatory? Does it differ?
Pupal Development Rate	Cohort experiment; Life Table analysis;	Is there a significance difference?
Pupal Survival	Cohort experiment; Life Table analysis; Population level modeling	Is it density-dependent? What is the type of density-dependence? Is it under/overcompensatory? Does it differ?
Adult Emergence	Cohort experiment; Life Table analysis;	Does the timing of adult emergence differ?
Mating Strategy	Cohort experiment;	Is there assortative mating? Are there costs to male/female gametes? Does the modification affecting mating competitiveness?
Sex Ratio	Cohort experiment; Life Table analysis	Is the sex ratio substantial different from the null expectation?
Flight Ability	Cohort experiment; Physiological experiment	Is flight duration or distance different?
Adult Size	Cohort experiment; Life Table analysis;	Is adult size different?
Biting Rate	Cohort experiment; Physiological experiment	Does the feeding rate differ?
Vector Capacity	Cohort experiment; Physiological experiment;	Is the capacity to harbor pathogens enhance/diminished?
Adult Survival	Cohort experiment; Life Table analysis; Population level modeling	Is it density-dependent? Is it enhance/diminished by the modification? Does it differ?
Insecticide resistance	Standard testing procedures	Is it expected to alter the competitive status of transgenic lines? Does it make transgenic lines less amenable to conventional control?

Table 3.2 Example parameters for open field studies and post-surveillance of transgenic mosquitoes. Risk assessment should build on Phase 1 and 2 trials and extend the hazard, the methods to measure these hazards and exposure assessments. Comparator studies aim to compare the GM mosquito with a conventional (non-modified) counterpart.

Parameter	RA Method	Exposure Assessment
Density dependence	Comparator studies; field trials; population-level modeling	Does the transgenic strain differ in the role of this ecological process
Spatial distribution	Comparator studies; field trials; population-level modeling	What is delimits the spread of the transgenic release? Is this appropriate? Is the spatial spread at an appropriate time scale?
Population size	Field assessment; population level modeling	What is the impact of the release? Is the release number appropriate?
Site selection	Field assessment; environmental-level modeling	Is the local environmental conditions (ecological, geological factors) appropriate for open-field trials?
Open field site size	Experimental design; field trials; statistical modeling	Is the proposed release at an appropriate scale? Is the chosen site appropriate to measure statistical and biological consequences of the release
Open field site number	Experimental design; field trials; statistical modeling	Is the proposed number of sites statistically and biological relevant?
Efficacy of spread	Comparator studies; Cohort-based field trials; Life table experiments; population-level modeling	Is competitiveness, longevity and/or dispersal different under field conditions?
Mass rearing	Cohort experiments; Comparator studies; Experimental design; pre-release surveillance; Post-release surveillance;	Does it affect mosquito densities, life histories and/or transgene stability?
Behavioral Resistance	Comparator studies; Cohort-studies; Post-release surveillance; population-level modeling	Under field conditions, what limits the spread of resistance due to mosquito behaviors? Is there potential for assortative mating in the field?
Biochemical Resistance	Comparator studies; Cohort-studies; Post-release surveillance; population-level modeling	Is the likelihood of resistance enhanced/ diminished in transgenic mosquito strains?
Vector capacity	Comparator studies; cohort studies experiment; Post-surveillance monitoring	Is the capacity to harbor pathogens enhance/diminished?

References – Section 3

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4. Ethics and Public Engagement

Summary: *Public dialog and outreach are important for realizing research goals, especially in the development of new technologies. Sincere and well-developed engagement can help to direct technical goals, reduce the chance of a misunderstanding of the science needed to meet the goals, and improve the performance of the research project in both technical and social contexts. Although engagement activities may overlap with regulatory requirements, researchers should not assume that regulatory compliance also implies that ethical and engagement responsibilities have been adequately addressed.*

Respect for communities should be an overarching ethical goal in GMM trials. Researchers will interact in the course of field testing with different public groups, ranging from those living within the trial site and directly affected by the conduct of the project to third parties interested in the research activities. GMM projects will have ethical responsibilities to people living within a trial site, even when these people are not, in a traditional sense, subjects of the research at hand. Researchers should initiate ethics and engagement efforts during Phases 1 and 2, in order to ensure that the goals and methods of the project are well defined and communicated, and meet genuine stakeholder needs. Internationally accepted standards for the participation of human subjects in research may apply under certain conditions in small trials with entomological endpoints in Phase 2, but will become more prominent in Phase 3 trials with epidemiological endpoints. Beginning in Phase 2 and expanding in Phase 3, community engagement activities are intended to address ethical responsibilities beyond the formal permissions required at the individual level (informed consent) and the governmental level (regulatory compliance). The concept of 'community authorization' entails providing those living in the trial site with methods to give or withhold agreement for trial activities, and to identify elements they believe to be important for the research to continue. During field testing, scientists also should expect to interact with third parties who express interest in the activity and its outcomes, both to ensure that the project is well understood and to avail the project team of information and insights that such interested parties might provide. However, given the diverse range and varied degrees of interest of third parties, there is not the same level of obligation to seek them out proactively to ensure that they are informed about the project, as is the case with those directly affected. In Phase 4, the responsibilities for implementing GMM technologies and interacting with affected individuals likely will shift to the relevant local, regional or national public health authorities.

There are aspects of ethics and engagement that may require special skills and training that biologists, medical personnel or public health specialists would not normally be expected to have. Engagement with people living within the field sites may require specialized knowledge of local culture and institutions. In addition, engagement with third parties may require broader communications and negotiation skills. Adequate time and resources must be allocated within the project plan to support these activities.

The success of scientific and public health endeavors can depend upon good will, cooperation and support from diverse sectors of the observing public. While members of the public certainly expect researchers to comply with regulatory requirements that govern the conduct of research, there is ample evidence that public expectations, and researchers' obligations, are not always satisfied simply by conforming to regulations and institutional policies. The word 'ethics' calls attention to concepts of right and wrong, and can imply a standard higher and more rigorous than that of civil authority. Regulations, laws and organizational policies dictate standards and procedures with which individuals and organizations must either comply or face sanctions that can range from warnings or admonishments to withdrawal of funding, fines and permission to operate or even to jail. In contrast to regulatory emphasis on compulsion and compliance, ethics can be understood as *activity* or *inquiry* whose purpose is to shed light on the correctness or justifiability of some conduct. In the context of GMM trials, ethics aims to understand the interests of stakeholders and their various entitlements, rights, other types of claims and obligations, including what actions or activities are required by the principle of respect for communities hosting the trials. Ethical questions include: How should these rights and interests be recognized in a decision for trials to proceed? How can researchers strike an ethically robust balance between the interests and rights of individuals, the collective interests of the host communities and the properly mandated activities of their public institutions? What is the appropriate role for communication and engagement with media, civil society organizations and others that take an interest in the research?

It is not easy to maintain a clear distinction between the activities of ethical reflection and engagement and those related to regulatory compliance, which have come to dominate the ethics of research with human subjects (Hagerty, 2004; Rollin, 2008). The major global agencies that fund GMM trials require compliance with international standards for research conduct, including submission of protocols for the use of human subjects, as well as biosafety and the use of animals, to appropriate regulatory oversight committees, usually as a requirement of their own domestic laws and regulations (Section 5: Regulatory Frameworks). This may cause confusion, since there is a common practice of referring to these obligatory requirements as "ethics" requirements and to various regulatory compliance bodies as "ethics" committees or boards. This section provides guidance for addressing broader ethical issues and responsibilities that are expected to arise in the context of GMM trials but are not specifically mandated by administrative law or organizational policies.

The Role of Ethics and Engagement in Science and Technology

Scientists have long appreciated the importance of public dialog and outreach to realize the envisioned results of their research. However, events and developments over the last three decades have led to a renewed interest in the ways and means for interaction between scientists and a number of distinct public groups with different attitudes and interests in regard to scientific work. Some of these events have cast science and technology in a heroic light, while others portrayed people in scientific and technical walks of life as lacking in moral sensibility or fellow-feeling. Others simply testify to the way that developments in science and technology can grip public attention, occasionally sparking reactions and consequences that the scientists involved never imagined.

The social phenomenon of public reaction to scientific developments has been the subject of numerous historical, philosophical and sociological studies. Ulrich Beck has argued that general public literacy in scientific matters has the ironic effect of creating a more sophisticated understanding of how

advances in the sciences are accompanied by both benefits *and* risks (Beck, 1992). As a result, citizens have become more aware of scientific or technical breakthroughs as potentially controversial. This awareness has been accompanied by the rise of numerous civil society organizations dedicated to promoting specific causes such as consumer rights, women's issues or environmental protection. The result is a greater willingness for citizens to become involved in promoting those scientific activities that they see as consistent with their values (such as poverty alleviation or protection of wildlife) or opposing technologies that they perceive to be against their values. Public resistance to certain agricultural and food applications of biotechnology, and to some specific applications of nanotechnologies, is seen as exemplary of this new awareness (Mcnaughten et al, 2008). At the same time, scientists themselves have become cognizant of new ways that involving non-scientists in their work can be beneficial. Exceedingly complex problems may require planned activities that engage non-scientists in collaborative or problem-solving roles, rather than considering them solely as subjects. This has led many to envision a new era of science in which many people can become enrolled in cooperative projects as "co-producers" of new knowledge (Haraway, 1989; Wexler, 2004).

Scientists undertaking work on the cutting edge of discovery or technological capability have both "positive" and "negative" motivations for paying attention to the reaction and receptiveness of the broader public. On the positive side, engagement with people not generally considered to be part of the research community can both enrich the research process and provide access to information and perspectives that would otherwise have been unavailable to people within the research group. It can also be instrumental for achieving the broader impacts that researchers seek. On the negative side, research that comes under public scrutiny can become the target of organized opposition that has the potential to frustrate not only the application of science, but even the research process itself. It will not be possible to avoid such opposition in every case. Sometimes opponents of science and technology are simply pursuing interests that are genuinely contrary to the advancement of a given technical project. Sincere and well-developed engagement that acknowledges and demonstrates respect for these perspectives may reduce the chance that opposition is based on a misunderstanding of the science or of its technical goals. In a more positive spirit, it can demonstrate respect for the communities involved in testing the new technologies and may sometimes result in changes or modifications to a research project that researchers view as beneficial.

It is especially important for scientists conducting studies likely to attract significant coverage from the media to consider how their work might be beneficially or detrimentally affected by rapid and broad engagement and interaction with members of the public who have no training in their disciplines or methods. Stories may be disseminated either through traditional media such as newspapers, television and radio, or through new outlets on the Internet and emerging social media. Ordinary word of mouth also can effectively spread a widely shared impression of research goals, intended applications and methods, especially within village or urban settings. Such broad representations of science can have the beneficial effect of expanding opportunities to obtain key informants, participants and partners. However, they also can spread misrepresentations, suspicion, distrust and antagonism to a scientific research project.

The Scope of Ethical Responsibilities

Respect for communities should be an overarching ethical goal in GMM trials. Although there is no consensus among research ethicists about what this requires in practice, the activities of community or public engagement may be best understood as opportunities for demonstrating respect for the

communities in question. Ethical engagement may begin with interactions within the project's scientific team. Scientists who are involved in work intended to benefit the public interest will typically engage in conversations and even debates over alternative ways to articulate the social goals that will be served by the research. This process of ethical reflection is an important component of ethical engagement, and projects moving to field trials should plan to devote time and resources to critical deliberative team activities dedicated to reaching and describing a common understanding of the ethical purpose and rationale of the research as an iterative component of the project plan. While this task will primarily be internal to the project personnel, over the course of the research it may include interactions with advisory committees and consultants, as well as other scientists whose opinions, views and reflections are sought on an *ad hoc* basis. As the project identifies candidate field trial sites, these reflective activities should be expanded to include critical deliberations with representatives from the host communities where the research may take place, and may include people from other interested groups in an advisory or consulting capacity.

Second, conducting research in host communities brings scientists into direct contact with a number of people, including, but not limited to, those who are research subjects or whose cooperation is necessary for successful completion of research tasks. Those conducting GMM research may be most familiar with the biomedical research model, where individuals who are the subjects of specific interventions or interactions, or from whom identifiable information, specimens or materials are collected are classified as "human research subjects" (see discussion below and in Section 5: Regulatory Frameworks). However, it is likely there will be additional individuals who do not fall within the typical definition of human subjects but who might still be affected by the conduct of research. This may include those living near a research project whose daily activities and/or livelihood could be influenced by research activities.

People living at a distance from the trial may have friends and relatives or even economic interests that they fear could be affected by the conduct of a research project, and thus also may perceive themselves to be affected by it. Moreover, a much larger community of people may take an interest in the conduct or outcome of research, even if they are unlikely to be physically affected by the trial activities themselves. For example, people who are afflicted with a particular disease (along with their friends and family) have an obvious interest in the outcome of research or clinical trials, even if they are not involved with specific trials. Such groups are likely to be strongly supportive of research intended to improve their condition. In a similar vein, people who care about causes such as protecting vulnerable groups or endangered species may take an interest in a wide range of research activities, and may not be unilaterally supportive of research goals or procedures. Although the nature of responsibilities to such individuals or groups is quite different from those to communities hosting the trial, it is clear that an effective plan for engaging a wide spectrum of interested parties can be critical to the success of research, especially for projects that can be expected to attract a significant amount of attention in the press or monitoring from civil society organizations.

The distinction between people who are affected directly by research and others who are more indirectly interested in its conduct may be operationalized in the way that the relevant ethical obligations are understood. For example, when research involves risks associated with organisms or substances released into the environment, as opposed to contained within experimental facilities, geographical proximity to the site of research becomes an important ethical indicator. In vector control projects, researchers have ethical responsibilities to people living within a trial site, even when these people are not, in a traditional sense, subjects of the research at hand. At a minimum, the engagement

that is ethically required includes an effort to identify the individuals at risk (even if risks are small). Depending on the governing regulations, some of these individuals in fact may be classified as research subjects, requiring individual informed consent and any other applicable protections, while for some other individuals investigators may meet their ethical obligations by procedures that would simply advise them that they may have interests at stake.

In the case of some GMM trials, defining the limits of potential effects may be complicated by the geographic mobility of both people and mosquitoes over time. Such considerations should have been taken into account in a risk assessment (Section 3: Biosafety), which will be helpful in guiding identification of stakeholders. Developing a set of criteria for discriminating between those individuals who are affected by the research activities through specific interventions or interactions, other members of host communities who have a stake in the trial, and those who may have legitimate but more distant interests at stake, and determining how to respond to ethical obligations in each case, will be a component of the broader ethical reflection needed by the project.

A Strategy for Ethical Engagement

The outline of a broad strategy for helping research teams to meet ethical responsibilities and conduct public and community engagement activities follows below. This will involve ethical reflection, interaction with the host community and a wide range of other interested parties, and iterative integration of findings from these activities into the ongoing planning and conduct of research. As such, the outline is to be interpreted as a description of processes and goals, rather than as a prescriptive formula. As noted by others, when "research ethics" becomes an activity of ticking boxes for compliance, or slavish adherence to rules, rather than one of thoughtful consideration, the real goals of ethical respect and responsiveness may well be lost (Hagerty, 2004; Rollin, 2008).

The ethics and engagement component of the research program can be visualized at three levels (Figure 1):

- At the project level, there are reflective tasks concerning the broader social and ethical issues raised by GMM trials that shape specific management goals and elucidate important learning and evaluation opportunities for the research. Such tasks are by no means unique to research on GMM. An explicit recognition and articulation of the ethical purposes of a scientific project is especially useful when the research is likely to attract public interest and scrutiny, as is often the case with a new technology.
- The researchers need to anticipate a set of tasks that arise from interactions and effects at the site(s) where field studies are conducted. These tasks overlap with, but are distinct from, regulatory requirements for securing appropriate informed consent and other relevant protections, and may also include involving and empowering local populations in key elements of research planning and implementation, as well as addressing both real and perceived issues that may arise in connection with the project, including broader socio-economic impact. These tasks may be thought of, collectively, as "community engagement."
- There will be tasks related to the involvement of individuals and groups who are *not* immediately affected by the research, including civil society organizations, the press and the general public. (Note that formal interactions with government authorities are addressed in Section 5: Regulatory Frameworks).

The plan for addressing each of these three levels of engagement should include activities appropriate for each level. Each of these activities should be understood as iterative and sustained during the entire research period, as illustrated by the feedback arrow loops in Figure 4.1. Each group of tasks should be understood as an ongoing component of the research activity, and the research plan should include a programmatic discussion of how tasks in each of these three areas will be carried out by members of the research team on an ongoing basis throughout all phases of the research activity. Although not depicted, researchers must take into account that communities and third parties may become engaged with each other independent of the project.

One helpful way to use the three levels of activities for planning purposes is to focus on *who* will need to be involved in completing them. Activities at all three levels of engagement involve participants from the research team, and will almost certainly involve staff from the sponsoring organizations as well. Meeting ethical responsibilities to the full range of stakeholders in the host community requires a great deal of work "on the ground" in the local areas encompassing the research field sites. As will be explained further below, this may not imply contact with literally every individual in the contiguous area, but it must be understood to require appropriate attention to local forms and mechanisms of representation for those who will be affected by the research activities. This may involve negotiation of the environmental and developmental goals, standards, and metrics for the research. For example, directly affected parties and international civil society groups alike may have a desire to participate in discussions about how risks to biodiversity are measured, or how economic benefits are understood in relation to improvements in public health. One cannot assume that any and all forms of economic growth or resource development will be seen as beneficial by all parties, and investigators should not assume that local communities will always be forthcoming or comfortable with expressing these interests. There may be some areas of overlap between the ethics issues that arise on the ground in interacting with local stakeholders and the ethics of environment and development that represent concerns of third parties. Some third parties might take themselves to represent the interests of local people, though the local communities may, or may not, view such representation as legitimate. Anticipating and preparing responses for the issues that are likely to arise in such interactions is an example of something that falls into the category of "broader ethical concerns" to be addressed at the project level.

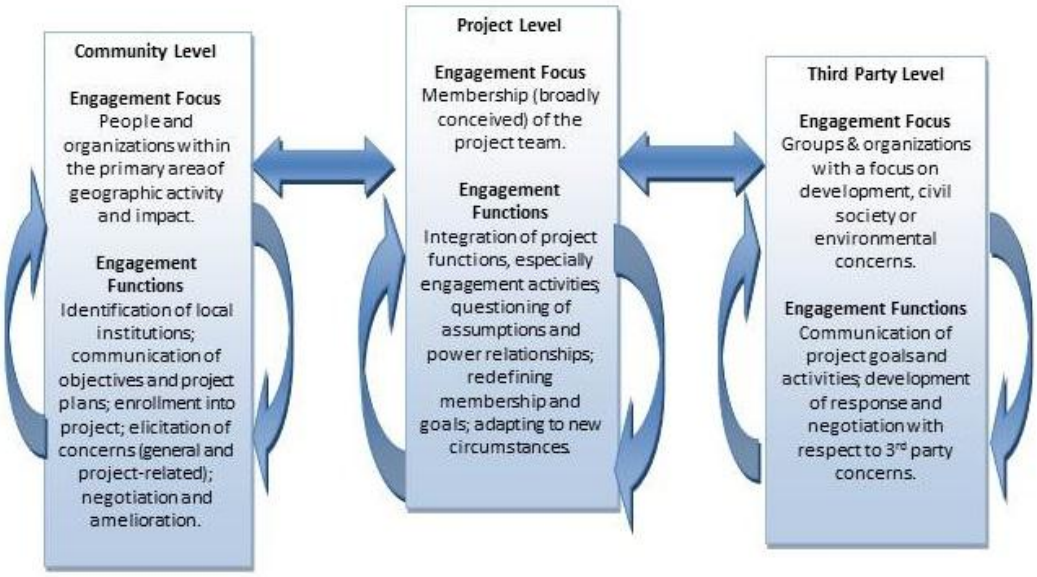

Figure 4.1 Levels of engagement focus and function

Activities at all three levels will include the following:

- ***Ongoing Literature and Methodology Development*** **-** Whether it be best practices for clinical and epidemiologic research, or engaging with communities, nongovernmental organizations or the press, there now is a body of relevant literature that should be taken into account in planning and implementation of a project of the scale required for GMM trials. Appropriate review and application of this information will require, at the project level, participation of team members or consultants with the necessary background and expertise.
- ***Task Planning and Implementation*** **-** Based on this literature, those responsible for the ethics and engagement activities will undertake the planning and implementation of project procedures. This may involve staff training, consultations, development of information about the project (including language and culturally appropriate information for use in interacting with residents at field sites), surveys, educational activities, workshops, negotiations, etc.
- ***Documentation and Reporting*** **-** Record keeping requirements are specified with respect to research involving human subjects. However, it must be stressed that other ethics and engagement activities conducted under the project also should be documented to allow for later reporting, and mechanisms should be developed to accomplish this. Records of ethical deliberations as well as stakeholder interactions and agreements could prove important in the case that challenges to the project arise. Reporting in the form of peer-reviewed articles on the ethics and engagement activities will enrich the literature and help with the planning of future GMM research.
- ***Evaluation*** **-** Both internal and external evaluations of how well tasks at each of the three levels are being performed should be part of the plan. Internal evaluations could potentially be done by one or more members of the project team, but the plan should specify such responsibility explicitly.

External evaluators can be drawn from project management specialists, as well as specialists in ethical dimensions of public health.

- ***Iteration -*** Evaluation should lead back to methods development and planning. This process will be repeated periodically as needed.

Activities at the project level

Most scientists view their work as having value and a social purpose, and this may be especially so for those conducting research on public health and disease control. However, scientists do not always articulate the purpose of their research explicitly, or discuss its value with others. Reflection is an activity of both articulating value and purpose and initiating critical discussion of the project among members of the project team. Reflective activities should encourage openness, among the research team, to the possibility that the social, medical or public health rationale for a project may not be sufficiently well-grounded to warrant its continuation. But more typically, these activities can stimulate constant reconsideration of project aims and research design and methods to ensure a continuous opportunity to bring project activities more fully in line with public health objectives and social goals.

Making explicit the value and social purpose of a scientific research project initiates a broader reflection that serves several key functions. First, an explicit discussion of how research will give beneficial outcomes can yield unexpected improvements in project design. Conducting such discussions with project team members, advisors, and consultants increases the range of knowledge and interests that can be incorporated into the research design, and will help to ensure that important strategies or alternatives are not overlooked. This helps researchers avoid losing time by pursuing strategies that may be technically feasible, but cannot be implemented due to their incompatibility with social mores, legal mandates or other elements in the technical infrastructure. Second, public presentations of a project's motivation, goals, and ethical vision and explicit articulations of the ethical considerations that guide the scientific work, and its relationship to various social goals, disseminates this thinking to a broader audience and may prove helpful in winning the trust and cooperation of host communities. Finally, the public record that is created by documenting how and why the science was done creates an opportunity for others to learn. Canada has pioneered approaches to embed such activities within large-scale research projects dedicated to biological research (Castle and Culver, 2006; Coward, 2006), and some of these may serve as useful templates for GMM trials.

It is especially appropriate for researchers working on GMM for disease control to engage in and support such reflective activities within their trials. There is a well-established record of conflicting views on the most appropriate strategy for addressing persistent global health problems such as malaria. Some authors express extreme skepticism about initiatives that propose "big science" approaches (Packard, 2007), as opposed to improved implementation of simpler and more accessible local solutions. Parties involved in GMM research should be aware of this history and be willing to reflect critically on the role of their own project in this enduring debate. Additionally, the use of GM approaches on animal species provides a second linkage of these projects to research traditions involved in well-established debates (Thompson, 2007).

Therefore, it is recommended that projects on GMM research include structured ethical reflection as a specific and planned activity, and allocate both time and resources to ensure that this is not neglected. It may be fully appropriate to schedule these activities in conjunction with key project milestones. These should incorporate some form of public reporting on thinking within the project,

including "lessons learned." Such public reporting might take the form of peer-reviewed publications in appropriate ethics or policy outlets, seminars or workshops, updates on the project website, etc. (for example, see Osrin *et al.*, 2009; El-Sayed *et al*. 2009; Lavery *et al*., 2010).

Activities at the host community level

People living at the trial site may be in immediate physical contact with the research team, their buildings and vehicles and with any materials or substances that are released, intentionally or not, into the environment. For GMM research, this includes the perceptions of people who may see, hear or be bitten by any mosquitoes in the field testing area. There may be some ambiguity in determining who has the potential to be affected in this sense, as there will be movement of both humans and mosquitoes through the locale and complex opportunities for different types of contact. Experience with GM crops illustrates the need also to consider possibilities for longer range economic, spiritual or cultural effects. Identifying who may be affected by a GMM trial, and in what ways, is itself a key project level ethics activity.

Human subjects research ethics

In the United States, the Belmont Report provided a framework of principles that aimed to clarify ethical obligations to human subjects in biomedical and behavioral research (U.S. Department of Health, Education and Welfare, 1979). These principles—respect for persons, beneficence and justice—provided the conceptual architecture for the current U.S. regulations governing research with human subjects. Similar regulatory provisions exist in most countries, and apply generally to research conducted under World Health Organization supervision or funding (Section 5: Regulatory Frameworks). These regulations require a process (known as "informed consent") intended to foster sufficient understanding to ensure that individuals participating in a research study are: a) fully and explicitly advised of all risks, costs or inconveniences they may bear as a result of participating as a research subject; and b) have voluntarily agreed to accept or bear those risks and costs.

Importantly, The Belmont Report, and the regulations its principles informed, have been criticized—among other things—for their lack of recognition that some research affects communities, and not just individuals (Levine, 1986). In this regard, an important feature of GMM trials is that the intervention (i.e., the release of the GMM in question) is intended to function at a population, rather than at an individual, level. This has important implications for GMM trials, since their effects will operate largely at the community level, and since it may not be clear how or when the activities that trigger the application of the regulations—intervention or interaction with individuals, or the collection of identifiable information about individual persons—are actually initiated, if at all, in GMM trials.

The implications for research ethics are potentially profound, since triggering the regulations through these activities entails a range of specific procedural requirements, including individual informed consent and review by a research ethics committee. Some of the issues with most immediate implications for GMM trials are: a) who is a "research subject/participant"?; b) under what circumstances, and from whom, is individual informed consent required?; c) how should the risks associated with GMM trials be characterized and communicated?; and, d) what constitutes adequate authorization from participating communities to conduct the trial?

Who is a "research subject/participant"?

As mentioned above, individuals living in the vicinity of GMM trials may not fit the definition of human research subjects, and yet they may be affected by the research. In a study of key ethical issues related to randomized cluster trials, a design that has been proposed for Phase 3 GMM trials (Section 2: Efficacy Evaluation) and has been used in the past to assess interventions aimed at populations rather than individuals, MacRae et al. suggest an interpretation of human research subject that they assert is consistent with a wide range of guidance documents (MacRae *et al.*, 2011). They define a human research subject as "an individual whose interests may be compromised as a result of interventions in a research study" according to four specific criteria - a human research subject is an individual: a) who is directly intervened upon by an investigator; or b) who is deliberately intervened upon via manipulation of the individual's environment by the investigator; c) with whom the investigator interacts for the purpose of collecting data; or d) about whom an investigator obtains identifiable private information for the purpose of collecting data (MacRae *et al*., 2011).

MacRae *at al*. further argue that "(f)or an individual to be properly considered a human research subject, the environmental manipulation must be designed to produce a direct effect on that individual." GMM are not designed to have a direct effect on people, but rather to reduce the numbers or alter the properties of resident mosquito populations. Thus, in the absence of evidence for a direct and potentially harmful impact on individuals, as determined by the appropriate risk assessments (Section 3: Biosafety), people living in the vicinity of GMM releases—according to MacRae's position—would not be considered *a priori* to be human research subjects, from whom informed consent would be required. Any risks associated with the GMM trial nonetheless should be communicated via the process of community engagement, as described below. Research subject status may arise, however, under the further criteria described by MacRae *et al*., as a result of interventions or interactions such as the collection of specimens, data and private information.

Under what circumstances, and from whom, is informed consent required?

These circumstances may arise in small entomologically-focused trials—for example, when individuals are approached to allow the collection of mosquitoes from within their homes along with addresses or other identifying information. Unless determined otherwise by the relevant institutional review boards or ethics committees, it may be presumed that informed consent should be obtained from such individuals in advance of the collection of data and this should be feasible. However, a number of researchers have noted that informed consent and cultural practices of individual decision-making and personal autonomy may be poorly supported in some situations. Mechanisms for providing information on risk may need to be tailored to local cultural practices and levels of linguistic and mathematical literacy (Shapiro and Meslin, 2001). Indeed, persons in some field areas may have little experience with anticipating hazards from technically-complex and uncertain research practices, and thus may be unwary and ill-prepared to participate in a conventional informed consent exercise.

How should the risks associated with GMM trials be characterized and communicated?

Since mosquitoes are capable of unpredictable movement among locations, it will be impossible, in advance, to identify all persons with whom they might come into contact. Indeed, in the general case of vector biology research it previously has been proposed that biosafety oversight (Section 3: Biosafety, and Section 5: Regulatory Frameworks) may be a more appropriate model (Aultman *et al*., 2000) than individual human subjects protection. Lessons may be taken from environmental health

programs, which typically involve risks that are unavoidable and as such not amenable to ethical procedures that presume an opportunity to exit or "opt out" of the risk bearing situation. Such risks are usually characterized in epidemiological terms that make it difficult to describe the exact causal mechanisms of exposure, or to translate population-based exposure calculations in terms that are meaningful to individuals. What is more, environmental risks raise ethical questions about the way that risks are distributed across economically, politically or ethnically vulnerable populations—problems of environmental justice. There are no ready analogs to environmental justice in standard human subjects research ethics (Lavery *et al*., 2003). Thus, research intended to better understand environmental health or that involves exposure to potential environmental hazards may need to be evaluated from an ethical perspective that incorporates considerations rarely contemplated within standard human subjects deliberations.

As the ethical evaluation of research places increasingly greater emphasis on the way that a research activity is intended to benefit the parties that will be exposed to risks (Emanuel 2003), it thus becomes increasingly important to involve and empower those parties. This does not undercut the rationale for an independent review process for research ethics, but it does require that the relevant processes include adequate representation from the host community. Needs will vary according to location and societal norms. In some instances, special measures and innovative organizational activities will be necessary, while in others it will be more important to work with well-established social and political procedures or institutions (McNaughton 2010). Greater attention to these processes in research ethics review can help to avoid circumstances in which host communities are simply passive recipients of activities designed and delivered by others (Crocker, 2008).

What constitutes adequate authorization from participating communities to conduct the trial?

Further ethical questions can arise in connection with the authorization or permission to conduct research. As described above, informed consent will be required of individuals who are deliberately intervened on in the course of a trial or interacted with for the purpose of data collection, in recognition of the principle of respect for persons. Similarly, it is anticipated that GMM trials will require formal authorization by relevant government authorities in recognition of the country's sovereignty (Section 5: Regulatory Frameworks). However, these two levels of formal permission may still not fully acknowledge the range of rights and interests at stake within the host community. Measures necessary to fill this gap can be thought of as being guided by the informed consent goal of protecting the interests of those who will be affected by research. But they may need to use new mechanisms for communicating the aims and methods of the science and the potential risks and benefits of the project, and for achieving sufficient assurance that the community has agreed that the research and public health interventions should take place.

Community authorization and informed consent share several key elements. Both promote a deliberative model for addressing ethical issues that arise in connection with research. Rather than relying on strict rules or criteria that must be followed, the deliberative approach mandates that ethical issues be considered before the research is actually undertaken and periodically reviewed. Both are intended as a mechanism for demonstrating respect for persons who will be affected by a research project or a public health intervention. Both imply "voice": an opportunity to express concerns and to receive replies that are addressed specifically to these concerns. A reply might take the form of assurances or clarification of activities and/or risks, yet for the conditions of voice to be met fully, affected parties must accept the assurances offered as a satisfactory response to concerns. Response also might involve modifications to the plan that relieve concerns, such as additions to risk assessment

or risk management activities. Community authorization and informed consent also are similar in that when the specified conditions have been met, the scientific team has received permission to proceed with its planned activities.

Community authorization differs from informed consent in at least three key respects. First, the methods of informed consent that have dominated discussion of research ethics in the industrialized world assume that consent is given or withheld by individuals. When possible, the individual in question is the person who bears the risks, but in cases of children or people who are incapacitated, a third party is authorized to give or withhold consent on their behalf. Community authorization is a procedure intended to elicit agreement on behalf of a group, often a political community such as a neighborhood or township. Procedures for community authorization thus more typically rely on norms for group decision-making such as voting, consensus or negotiations with leaders and representatives who are recognized as having the authority to speak on behalf of the community as a whole. Since norms for group decision-making vary widely, it is especially critical that procedures for identifying leaders and representatives, or for interacting with community groups, are based on detailed knowledge of the locale, its traditions and its history of cooperation, exploitation and conflict resolution (Christakas, 1992).

Second, even where there are established leaders and decision-makers in the host communities, trials of GMM are likely to involve a wide range of interests spread across a number of different groups, not all of which will be governed by the same leaders. As a result, researchers should be wary of assuming uncritically that any one decision-maker can provide definitive representation of a host community. One key implication for authorization is that, unlike individual informed consent, there may not be one specific mechanism or point in time in which authorization is granted. Instead, it is likely to be more of a judgment on the part of researchers that they have exercised the appropriate level of diligence in eliciting and responding to the concerns of the interested parties and groups, and vigilance in maintaining the necessary commitments and relationships once it is determined that there is a general collective will to proceed. In the absence of a specific mechanism, authorization may represent an accumulation of endorsements or assent by key stakeholders. These activities, collectively, and sustained over the full duration of the GMM trial, from planning to post-trial negotiations, constitute community engagement, which is described in more detail, below.

And third, unlike individual informed consent in most biomedical research trials, community engagement and authorization by the community for GMM trials will likely not be sufficient, on their own, to allow trials to proceed. There will usually be a need to secure formal government permission to import the GMM to be used in the trials and to begin the planned trials (Section 5: Regulatory Frameworks).

Community Engagement

Engagement and involvement with the communities hosting the GMM trials must be guided by detailed knowledge of the local community, its institutions and common practices. Finding out what kinds of concerns the community might have, any past engagements around science that went badly, or determining what the community wants/expects in terms of engagement or consent will be important (McNaughton, Clough *et. al.* 2010). In most cases, such information can only be gleaned from extended ethnographic work and ongoing relationships with individuals from different social classes, gender, occupation and social role. It is only in light of empirical findings and the establishment of the necessary

relationships that will be unique to each setting in which GMM trials will be conducted that an appropriate process of ethical review and engagement can be conducted (Marsh *et al.*, 2011; Hyder *et. al*., 2009). In many cases, particularly in more traditional community settings, community leaders may play a central role in introducing the researchers to the community and its social structures (Tindana et al., 2011) and in providing various levels of ethical scrutiny and permission (Diallo *et al*., 2005).

Community engagement, at the most general level of description, is a set of procedures and their motivating ethical goals that aim to develop fair and respectful collaborative interactions with communities around the introduction of a new technology or intervention in a way that protects the interests of the community while permitting the introduction and testing of promising new technologies to improve health. Although detailed guidance about what constitutes effective community engagement is still under development, one of the first frameworks for community engagement in global health research was developed specifically for trials of GMM (Lavery, Tindana *et al*. 2010), and so presents a potentially very useful resource for the design of community engagement activities to support the type of authorization from host communities described above (Box 4.2). This study also addressed the issue of how to define the community for purposes of engagement, citing two principles: 1) the community comprises at least those individuals who share identified risks associated with the proposed research project; and 2) there may not be a pre-existing and established community in the way envisioned by the researchers, but rather, the relevant community may take form progressively in response to specific aspects of the research and to engagement activities associated with the project (Lavery, Tindana *et al*. 2010).

Box 4.2. 'Points to consider' for effective community engagement:
(i) Rigorous site-selection procedures
(ii) Early initiation of community engagement activities
(iii) Characterize and build knowledge of the community, its diversity, and its changing needs
(iv) Ensure the purpose and goals of the research are clear to the community
(v) Provide information
(vi) Establish relationships and commitments to build trust with relevant authorities in the community: formal, informal and traditional
(vii) Understand community perceptions and attitudes about the proposed research
(viii) Identify, mobilize, and develop relevant community assets and capacity
(ix) Maximize opportunities for stewardship, ownership, and shared control by the community
(x) Ensure adequate opportunities and respect for dissenting opinions
(xi) Secure permission/authorization from the community
(xii) Review, evaluate and if necessary, modify engagement strategies

From Lavery *et al*., 2010.

Within the community engagement framework proposed by Lavery *et al*. (Box 4.2), items ii)-x) address specific needs for information or activities that will almost certainly need to be supervised by persons

with training in appropriate field-disciplines in the social sciences. Persons who are naturally fluent in language, local tradition and customs and who translate between the community and a research team while effectively communicating risk are rare. Furthermore, these individuals will need to budget a significant time commitment to activities *in situ* with local communities, and these activities will require a significant budgetary commitment from the project. The composition of the research team should reflect the process for engaging with local communities, gathering this information and integrating it into the project's planning and deliberation process. Depending on competencies of both project staff *and* locally affected parties, it may be appropriate to include representatives of affected groups within the project's governance mechanisms.

Activities at the third party level

Those with interests in GMM trials probably will not be limited to individuals and households with the closest geographic proximity to the trial sites. Instead, there may be a wide range of individual and groups that have a legitimate interest in the conduct and outcomes of the trials. Relevant 'third parties' may include the following groups:

- Persons associated with global or regional public health and international development organizations, including governments.
- Scientists and members of scientific organizations with disciplinary or trans-disciplinary links to research activities associated with field testing activities, including sciences dedicated to public health and infectious disease.
- Persons and organizations engaged in competing approaches to control of infectious diseases.
- Members of organizations focused on promoting the interests and protecting the rights of poor and/or historically marginalized people.
- Members of organizations dedicated to the preservation of endangered species, genetic diversity and threatened ecosystems.
- Members of organizations with a history of monitoring the role of the sciences in debates over the use of biotechnology.
- Individuals and organizations with ties to national, regional and cultural groups active in the areas where field testing is occurring.
- International organizations such as those within the United Nations system.

Some of these groups and the individuals involved with them may have either formal or relatively well-established ways to express views on GMM projects intended for controlling disease vectors and to interact with project staff, while others may not. In light of experiences with the global controversy over GMOs, it is wise from both an ethical and a strategic perspective for any community engagement framework to include mechanisms and procedures for engaging with third parties in a systematic fashion.

There is not the same level of obligation to seek third parties out proactively to ensure they are informed about the project as is the case with those that may be affected by virtue of proximity to a trial. However, interaction with third parties is ethically responsible because the parties listed above have legitimate interests in the conduct and outcomes of GMM field testing. In order to fully satisfy the ethical requirement of respect for the relevant communities, the project team must develop and implement planned activities for considering the interests of third parties, for engaging with third parties in a respectful manner, and for determining when duties to consider the interests of third parties

or to involve them in project decision-making or oversight are overridden by more compelling concerns or ethical responsibilities. Engagement with third parties could become so consumptive of time and resources as to hamper other aspects of the project. The ethical responsibility to inform and engage third parties must be balanced against the need to utilize time and other resources in completing overall project goals. Undertaking a process of stakeholder analysis early in the project may be helpful in this regard, by facilitating the identification of third parties most likely to influence the success of the project (Bryson, 2004).

In addition to being ethically responsible, engagement with third parties may be of strategic importance to the project's success. Third parties may have information or comments that can materially improve project activities. Their support and good wishes may contribute to a variety of activities ranging from securing funding or regulatory approvals to facilitating interactions with other scientists, suppliers, publication outlets and local officials. Strategically-motivated interactions with third parties are an inherent part of science (Latour, 1987; Collins and Pinch, 2002) and should not be regarded askance. Scientists are adept at some strategic interactions, especially those relating to their disciplinary colleagues, but can be inept at others. According to Dan Charles' history of agricultural biotechnology, many avoidable misunderstandings and much mistrust occurred because scientists in both public and private sector positions were insensitive to the fact that consumers and environmental advocates perceived themselves to have legitimate interests that were being neglected in the process of developing transgenic seeds and animal drugs (Charles, 2001). What is needed for strategic management is a broadening of the perspective that scientists bring to their research to include an effort to understand and then interact with people holding perspectives on the research project that may seem initially to be unrelated to, or at odds with, those of the scientific team.

The mechanisms for accomplishing this kind of broader outreach and engagement are still not well understood. One lesson that is now well established is that this kind of activity should *not* be conceptualized solely in terms of "public education", or of simply informing third parties of things that the researchers know about GMM and vector control. Communications launched with this so-called "deficit model" of public engagement have been shown not only to fail, but to substantially increase opposition and mistrust, (Klienman, Eisenberg and Good, 1978; Wynne, 1996; Hansen *et al*., 2003; Gjerris, 2008; Toumey, 2009). Rather, it is crucial to develop mechanisms of interaction with third parties that are based on what Pielke calls "the honest broker" approach (Pielke, 2007). The keys to this approach are to first recognize that third party interests reflect values-based standpoints that inform the way that a scientific research project is going to be seen as either responsive to a problem, or alternatively as contributing to a problem. Second, it is critical to develop communication materials about the project that are framed in response to these values-based perspectives. Putatively "neutral" descriptions of projects may fail to provide information that allows third parties to gain a clear understanding of why the research is relevant to them. If such materials are disseminated to parties that are already suspicious or skeptical of a project, they can actually exacerbate feelings of mistrust. Finally, it is important to present a picture of the research that includes both strengths *and* weaknesses relative to the values-perspective that would motivate a third party to take an interest in it. While such a communications strategy should strive to be complete in its accounting of strengths and weaknesses, it should also be sensitive to the need for a concise treatment focused on the problem at hand.

Thus, projects should include a general communications strategy based on Pielke's principles (Pielke, 2007). These communications can be disseminated through an array of media, including the Internet and through presentations at professional or public meetings relevant to key interests (e.g.

environment, public health, poverty and development, science policy). Other strategies for engagement with the public utilize universities, television and science museums (Wilsdon and Willis, 2003).

Once a public engagement strategy has been launched, there should be opportunities for follow-up activities. These could include provision for the submission of comments and questions, but might also involve more extended interactions. It is crucial that third parties invited into engagements of this sort are not made to feel that they are being placated, simply tolerated or even worse, that the engagement is simply a stalling tactic with little genuine opportunity for third parties to have any substantive input (for example, Griffiths and Steinbrecher, 2010).

Just as with discharging responsibilities for engagement with those immediately affected by research, engagement with third parties will be more effective if researchers and/or consultants with specialized skills are part of the project team. As such, there should be a component of the research activity that is designed and dedicated to third party engagement. It should be equipped with adequate personnel and budget, and this should include some time and energy commitments from leaders in the biological science component of the research. This is an important point for funders of GMM trials to understand, as these types of communications activities are not a standard component of research budgets.

When Should Ethics and Engagement Activities Take Place?

The timing for tasks such as securing authorization and support from those that will be affected by the research likely will be implicit within the nature and goals of the activity. It is important to stress that these procedures must be organized and conducted in advance of actual impact on affected parties. However, agreement secured too far in advance will simply need to be renewed, as people do change their minds. Similarly, there will need to plan efforts to revisit these tasks over the course of the project.

Phase 1 – 2 Trials

The traditional model of engagement and outreach for scientific research that held sway for the first half of the 20th century would have envisioned little need for engagement activity at the early stages of research, up to and including field testing for agricultural or public health interventions. According to this view, the public did not need to be particularly aware of a research activity until their help or cooperation was needed in actually undertaking a large-scale intervention. However, as cognizance of risks to human research subjects grew and standards for procuring cooperation and consent began to develop, researchers recognized that there were key activities needed to inform and involve affected parties, even at this relatively preliminary stage of research. While it is less likely that major controversies would erupt before field testing, the complexity of GMM research suggests that it is advisable for researchers to commence the “broader issues” engagement component as early as possible, and certainly before Phase 1 proof-of-concept work has been completed. This could be done, for example, by collaborating on a publication that discusses the ethical rationale behind proof-of-concept work. Need for stakeholder engagement and community authorization activities would be expected to arise in Phase 2 of the proposed GMM testing pathway.

A few key episodes in field testing have demonstrated how poorly executed public relations and engagement strategies can sabotage research efforts, sometimes having extremely long-lasting effects. Particularly relevant to field tests for GMM is an episode that occurred in conjunction with a field

release of male sterile mosquitoes as a component of research on vector control in India (Box 4.3). A cooperative project involving scientists from India and the United States, among others, was conducting field trials with male sterile mosquitoes as basic research that could be adapted to a number of disease control situations. However, suspicions were raised both locally and in the national press about the nature and intent of this research, which were exacerbated by poor communications, and the project was unable to continue (Anonymous, 1975). This episode has been repeatedly cited by those who warn that field tests for GMM must be accompanied by effective efforts to engage both local individuals in areas where field trials will be conducted and also activists self-identified as promoting pro-poor, pro-environmental issues and democratization of science initiatives, (Benedict and Robinson, 2003; Curtis, 2006; Knols et al, 2007).

Box 4.3: Disruption of the testing of male sterile mosquitoes in India
Public health scientist Robert S. Desowitz described an episode in one of his books written for a popular audience that is instructive to consider: "On a morning in 1975, a van bearing the blue-and-white logo of the World Health Organization on the door—a snake caduceus through a global map—drives into the village center. The villagers, who have a fear and loathing of snakes, regard the serpent van suspiciously. They begin to be even more suspicious when a peculiar collection of men emerges from the van—a few undoubted Indians, some strange Orientals, and some very white white men. An angry murmur of astonishment passes through the gathered group of villages when these men remove large mesh-covered cages from the vehicle, open the cages—and out flies a cloud of mosquitoes. Without a word of explanation, the snake and mosquito men then return to their vehicle and drive away. Several weeks later, the snake van appears again in the village and once more the strange foreigners release a cloud of mosquitoes from the cages. The crowd reacts—chasing the men into the van, which makes a hurried escape. A month or so later the vehicle appears again. The villagers burn it, (Desowitz, 1991, p. 89)."
Desowitz writes that the villagers complained to Parliament, and that Parliamentarians accused the American scientists of being agents for the CIA who were conducting an experiment in biological warfare. It was later revealed that suspicions of research on germ warfare were entirely unfounded (Powell and Jayaraman, 2003).

These incidents illustrate why adequate plans for communication and engagement are important even at the early field testing stage. This brief history of unfortunate episodes testifies to the potential for misunderstandings that can cause irreparable damage to specific research efforts. What is more, knowledge of these cases inclines some public advocates to be highly skeptical of the intentions and ability of scientific research efforts to respect and involve an appropriate cross-section of stakeholders, affected parties and representative members of the interested public through key phases of planning and executing field trial activities. While engagement activities complement and support other project activities that are dedicated to the anticipation and management of risks or regulatory compliance, the history of field trials gone wrong shows that these components have a purpose that is independent of risk management and regulatory compliance. Protecting the integrity of the trial and the ability to work both locally and in a global culture of support for the project depends on a good faith effort to engage social and ethical issues.

It is recommended that investigators work cooperatively with their institutional committees, including committees responsible for research ethics review, and with the host communities to avoid miscommunications and misunderstandings that could undermine trust and transparency. When field releases begin, communications should be careful to explain that trials are research activities intended to test the efficacy of a new technology, a protective effect is not assured, and the community must continue to employ other available methods to protect themselves from disease transmission.

Additionally, as described above, certain individuals may meet the criteria of research subjects, even in the case of small entomologically-focused Phase 2 studies, as a result of interventions or interactions such as the collection of specimens, data and private information. Unless determined otherwise by the relevant institutional ethics committees, it may be presumed that informed consent should be obtained from such individuals in advance of the collection of data.

Phase 3

Efforts to engage potentially affected parties will expand in anticipation of larger Phase 3 trials. In addition, human subjects issues will become more prominent, especially in trials seeking to evaluate epidemiologic efficacy where measures of infection incidence and other medical information will be required. Such trials are likely to assign groups of individuals to treatment and control clusters, rather than to involve a randomized distribution of individual subjects. Some individuals in clusters may have no direct contact with researchers, and their personal identities may not be relevant to the research process. For these individuals, the above argument that they are not subject to a direct affect of the research can be made. However, in Phase 3 trials for epidemiologic endpoints, data collection designed to shed light on the health impact of GMM releases will require the selection of individuals within the community for the purpose of securing the necessary data or personal information, for example through surveys or blood samples. Even in large-scale trials, these procedures would resemble those of vaccine trials, which typically require multiple interactions with individual participants over the course of the trial, and which also provide appropriate contexts and moments for securing and reaffirming informed consent. An important difference between GMM trials and vaccine trials is that in GMM trials, participants would be consenting (or not) to the collection of data, *not* to the intervention itself (the GMM release), which would not affect them at an individual level.

It has sometimes been the case in cluster randomized trials of the type envisioned for GMM trials, that the consent of the relevant cluster population(s) has been sought from a "guardian," such as a village elder or elected official, and perhaps without the knowledge of those involved in the trial, in order to avoid the possibility of changing behavior or otherwise biasing the control cluster (Edwards, Braunholtz, *et. al.* 1999). An ongoing metastudy of such trials suggests that ethical issues have not been sufficiently clarified, and that ambiguities leave open the potential for ethical abuse with respect to the level of understanding and agreement that is required from the study population (Weijer, Grimshaw *et. al.* 2011). As described above, appropriate community engagement and community authorization procedures would be expected to adhere to the principle of respect for communities, aiming for widespread understanding and on-going endorsement by those living at the trial site.

Another question that will be encountered in Phase 3 trials concerns the type of care that should be provided to control groups during a randomized controlled trial. The ethical debate generally centers around whether the control group should receive a 'proven effective' treatment, the 'locally

available' treatment, or some other treatment (van der Graaf, 2009). It is not clear that "standard of care" is even an appropriate concept for GMM trials, since the concept has been imported uncritically from drug and vaccine trials which are different in several ethically relevant ways. However, it is likely that research ethics committees will require investigators to design trials to ensure that other forms of vector control, or other treatments that reduce the amount of human infection, and could therefore influence the background level of pathogen transmission, will be provided. This type of requirement could have significant impact on trial design, since low transmission levels will make the efficacy of GMM more difficult to measure. For example, in trials of GMM for malaria control, one ethical question might be how actively to promote the concurrent use of bed nets. Another such question will arise if/when a malaria or dengue vaccine becomes available for public health intervention.

Further work will need to be done to determine the most appropriate way to conceptualize these 'standard of care' issues for GMM trials, but as these specific aspects of ethical trial design are being developed, investigators should prepare appropriate strategies for addressing such issues, along with the rationales for adopting them. These will prove to be useful for research ethics review committees and constitute an important aspect of the ethical reflection activities, described above. As noted in the Section 5: Regulatory Frameworks, requirements for regulatory compliance will be established by the appropriate governmental or institutional bodies. A robust ethical inquiry informed by a current understanding of the literature on trial design, relevant precedents, and current government policy at field sites will enhance a GMM research group's ability to develop appropriate protocols and anticipate the concerns of regulatory authorities.

Phase 4

Should GMM strategies mature into widespread public health initiatives, the responsibilities for implementing these technologies will likely shift to the relevant local, regional or national public health authorities. Controversy over the fluoridation of public water supplies, regulation of tobacco use and vaccination testifies to the fact that it is not unusual for public health interventions to be undertaken without the explicit approval of all affected parties (Cassidy, 2007; Powles, 2009). They nevertheless have legitimacy when conducted within proper democratic processes and institutions and with proper mandates. Any public health initiative takes place within the context of legal, regulatory and political institutions that are intended to resolve differences of opinion and to negotiate matters concerning who bears what risks. When public health authorities and the relevant ancillary institutions are functioning well, the responsibility to engage with affected individuals will likely be transferred to them once it has been established that the technology is safe and effective. In cases where local or regional institutions are not functioning well, researchers and sponsors may have additional responsibilities related to capacity building and planning with host country agencies and for maintaining the relationships of trust that were established through the course of the earlier phases of the trials.

Who Should Undertake Ethics and Engagement Activities?

The activities described in each section of this guidance framework are material to the successful accomplishment of research objectives. As such they should involve lead researchers and also will often require attention from other members of the project team who are focused on specific tasks. However, there are aspects of each element that may require special skills and training that biologists, medical personnel or public health specialists would not normally be expected to have. As noted above, engagement with affected parties may require specialized knowledge of local culture and

institutions. In addition, engagement with third parties is increasingly characterized as requiring skills for creating, maintaining and managing the forums in which discussions, consensus seeking and negotiations can take place (Dietz *et al*., 2003; Bäckstrand 2004). The abilities and methods for accomplishing these tasks are themselves the focus of ongoing research in communications and governance activities (Brown *et al*., 2010). It will be most expeditious for project directors and managers to consult or contract with specialists who can accomplish specialized elements of the ethics and engagement plan. Skills and methods are under constant development (Kreuter *et al*., 2004; Brown *et al*. 2010). Allowance must be made in the project budget for these types of activities. However researchers should not presume that they can simply turn the ethics and engagement component of the project over to a contractor, as the involvement of project leaders in ethical reflection and engagement and communication regarding research goals and conduct is vital.

Capacity Building Goals

Projects likely will discover a need for additional training of entomology researchers about ethics obligations in vector biology research. Likewise, there may be a need to train bioethicists and social scientists involved in the project about the unique situations encountered in vector biology research. As discussed above, this is a complex subject where the internationally accepted standards developed for clinical research are not always directly or clearly transferable. Additionally, there may be a need to train institutional and national ethics review committees on the importance and process of ethical review of GMM trials. In both developing and developed countries, ethics review committees often lack vector biologists and awareness of ethical issues in entomological research protocols/proposals. Attempts should therefore be made to create awareness of such issues among committee members responsible for approving and providing oversight for the planned trials, and to encourage the committee to seek appropriate expertise when considering GMM research/trials.

References – Section 4

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5. Regulatory Frameworks

Summary: *Regulation of GMM will control their release into the environment and transboundary movement through the laws and regulations of a nation, state, province, county, or lesser levels of jurisdiction. There are a number of GMM regulation types, options, and levels that exist and may have to be addressed during GMM development, including: institutional biosafety and ethics committees; laws and regulations governing human and animal pests, diseases, and drugs; laws and regulations pertaining to mosquitoes and threatened, endangered, and protected species in respect to biodiversity; and new laws and regulations under development specifically for living or genetically modified organisms (LMOs or GMOs). An important resource for specific country regulations and contacts relevant to GMM is the Cartagena Protocol on Biosafety, Biosafety Clearing-House.*

Regulatory agencies will be involved at most phases in the research and development process for GMM and may also be involved in post implementation surveillance. The mechanisms of regulation may include institutional biosafety and ethics committee approvals, risk assessments, public comment periods, and permits for importation and quarantined experiments, and may involve official review by more than one regulatory agency.

Multinational corporations have established precedents involving environmental risk assessment and regulation in their development and marketing of LMO crop plants. These costly and lengthy processes may be justified by profit incentives and the exclusiveness of data generated for regulatory approvals of agricultural products. However, they could present a substantial financial barrier to development of GMM for public health uses. Therefore, there is a need for regulatory agencies to define science-based, case-by-case targeted requirements with a degree of practical parsimony for GMM, which acknowledge the potential health benefits rather than relying on a precautionary approach.

Regulation of GMM is useful both for the scientists involved in their development and for the general public, because it provides a recognized and respected mechanism for protecting human health and rights, livestock, economics, and the environment. A thorough, science-based GMM regulatory process that is publicly transparent, without conflict of interest, contains minimal confidential business information, and provides allowance for public stakeholder input, will serve to strengthen public confidence in and acceptance of GMM biotechnologies, their developers, and the government agencies that regulate them.

Regulation of GMM will control their release into the environment within sovereign states as well as their transboundary movement. Pertinent developments are recorded in Table 5.1. Precedents exist from the regulation of other technologies, including other GM insects of agricultural importance that can inform the formulation of regulatory pathways for GMM. However, differences in the goals and funding resources between GMM and other GM technologies must be taken into consideration in order to avoid the inadvertent creation of unconstructive roadblocks to the development of a potentially useful public health tool. Nonetheless, such considerations must not compromise safe use of GMM.

The Purpose of Relevant Regulations

A regulation is an official rule to manage the conduct of those to whom it applies. Regulations are usually developed from legal interpretations of enacted legislation, laws, or acts of a legislative body and are implemented by government ministries or agencies under the authority of legislation, a law, or act. Regulation may be through the laws and official codes of a nation, state or province, county, municipality, tribe or other jurisdictional unit, and/or under the authority of laws and regulations enacted through provisions of a treaty ratified by participating states. A regulatory agency (also called regulatory authority, ministry, regulatory body, or regulator) is a public authority or government entity responsible for exercising autonomous authority over some area of human activity in a supervisory capacity.

The purpose of a regulatory agency in regard to GMM is to ensure that the safety of the public and environment are protected against risks or damage. Risk, and sometimes benefit, assessments (Section 3: Biosafety) are essential components of the regulatory process. A benefit assessment for GMM is that of performance or efficacy values for vector and vector borne disease reduction, without which there would likely be increased or continued risk of disease in the absence of alternative effective interventions. Although performance or efficacy, safety and risk assessment, and public transparency, communication, and acceptance are subsumed as part of the regulatory process, they are not covered in this chapter since they are discussed elsewhere in this guidance.

Government agency regulation of GMM could involve more than one regulatory authority and require more than one permit or license for importation of and research on a GMM. Examples of potentially relevant regulations illustrate this issue.

Biosafety

Institutional biosafety committees (IBCs) are charged by certain laws with the planning and implementation of university and other research facility biosafety programs for the purpose of protecting health and safety of all personnel working with potentially hazardous agents. IBCs may not exist in at an institutional level in some countries; they may be national or may exist at local, regional, state, provincial, or territorial levels of government. Where they do not exist, they should be part of capacity building by international or foreign aid organizations. IBCs may also draft institutional biosafety policies and procedures and review individual research proposals for biosafety concerns. Concerns relevant to GMM may relate to safe handling of recombinant DNA or pathogens perceived to pose a health threat. For example, in the USA, an IBC insures that research conducted at an institution is in compliance with NIH Guidelines for Research Involving Recombinant DNA Molecules[54] and the select agent regulations under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, which authorized regulation of the possession, use, and transfer of select agents and toxins. The USA National Select Agents Registry (NSAR) (USDA, APHIS and US DHHS, 2010) includes disease agents transmitted by mosquitoes, but does not include *Plasmodium* spp. or dengue virus serotypes. The NSAR currently requires registration of facilities including government agencies, universities, research institutions, and commercial entities that possess, use, or transfer biological agents and toxins.

[54] http://oba.od.nih.gov/rdna_ibc/ibc.html

Human Subjects

In research, human subjects regulations generally apply when data will be obtained from living individuals through an intervention or interaction, or identifiable private information will be made available. For example, in GMM trials, human subjects regulations would apply to the taking of blood specimens to measure epidemiologic endpoints (an intervention) or personal opinion surveys to understand concerns about the research (an interaction) as referred to in Section 2:Efficacy Evaluation and Section 4:Ethics and Public Engagement, respectively.

Box 5.1 In medical research, the "Nuremberg Code" from Trials of War Criminals before the Nuremberg Military Tribunals set a base standard following 1947 (Nuremberg Code, 1949). There are ten points concerning informed consent that are described in the Nuremberg Code and are in National Institutes of Health, Directives for Human Experimentation (NIH, 2010). The Declaration of Helsinki was issued by the World Medical Association (WMA) as a set of ethical principles for the medical community regarding human experimentation (WMA and NIH, 1964-2004). The Declaration is not a legally binding instrument in international law, but instead draws its authority from the degree to which it has been codified in, or influenced, national or regional legislation and regulations. The Declaration more specifically addressed clinical research under the term "human experimentation" used in the Nuremberg Code. The operating principles of the Declaration are the following: Research should be based on a thorough knowledge of the scientific background (Article 11), a careful assessment of risks and benefits (Articles 16, 17), have a reasonable likelihood of benefit to the population studied (Article 19), and be conducted by suitably trained investigators (Article 15) using approved protocols and subject to independent ethical review and oversight by a properly convened committee (Article 13). The protocol should address the ethical issues and indicate that it is in compliance with the Declaration (Article 14). Studies should be discontinued if the available information indicates that the original considerations are no longer satisfied (Article 17). Information regarding the study should be publicly available (Article 16). Ethical responsibilities extend to publication of the results and consideration of any potential conflict of interest (Article 27). The interests of the subject after the study is completed should be part of the overall ethical assessment, including assuring their access to care (Article 30). Wherever possible, unproven methods should be tested in the context of research where there is reasonable belief of possible benefit (Article 32). The International Covenant on Civil and Political Rights (ICCPR, 1976) is a multilateral treaty adopted by the United Nations General Assembly on December 16, 1966, and implemented March 23, 1976. It commits its parties to respect the civil and political rights of individuals, including the right to live, freedom of religion, freedom of speech, freedom of assembly, electoral rights, and rights to due process and a fair trial. As of October 2009, the Covenant had 72 signatories and 165 parties. The ICCPR is part of the International Bill of Human Rights, along with the Universal Declaration of Human Rights (UDHR) and the International Covenant on Economic, Social and Cultural Rights (ICESCR), of which Article 7 states the following: "No one shall be subjected to torture or to cruel, inhuman or degrading treatment or punishment. In particular, no one shall be subjected without his free consent to medical or scientific experimentation."

Institutional ethics committees (IECs), also known as institutional review boards (IRBs) or ethical review boards, provide oversight for biomedical and behavioral research involving humans with the aim to protect the rights and welfare of research subjects. Human subjects regulations and IECs were developed in response to notorious abuses carried out in the past in the name of research (see Box 5.1).

One role of IECs is to attempt to ensure that human participants in a clinical study understand the facts, implications, and consequences of their participation. Informed consent is the mechanism usually used for this purpose. Informed consent is intended to be a process of communication between an individual contemplating taking part in a study or trial and the physician or scientist administering the study, which results in the patient's decision regarding authorization or agreement. The most important aspect of informed consent is voluntary agreement. In order to give informed consent, the individual concerned must have adequate reasoning faculties and be in possession of all relevant facts at the time of consent. Countries will vary in regard to laws and regulations governing standards of informed consent that are required under common law and

statutory authorities, and in some countries informed consent statutory requirements may not exist or not be enforced. The components of informed consent have been delineated in many venues.[55]

GMO regulation

Mosquito pests

The intent or purpose of introduced genetic traits in mosquito population suppression could possibly be considered and regulated under the definition of a biopesticide when a pesticide is defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, which in the USA is the Federal Insecticide and Rodenticide Act (FIFRA) definition. Other national pesticide legislation may regulate on the same basis of pesticidal intent.

Mosquitoes are pests of livestock as well as humans. Many countries developed legislation to prevent and control outbreaks of livestock pests and diseases, the same as legislation existing for crop plant pests and diseases, as these issues are in the economic interests of most countries. LM or GM plants are being regulated under legislation intended for the protection of crops under the Plant Protection Act (PPA) of 2000 in the USA. GM *Drosophila* have been subject to importation and interstate movement permits under this act and more movement permits for genetically modified *Drosophila* have been issued than for all other GMOs combined. GMM have also been moved and tested in quarantine containment facilities under these same kinds of permits as a courtesy to GMM science and scientists to facilitate their research.

GMM might have been regulated in the USA under the Animal Health Protection Act (AHPA) of 2002 because mosquitoes are livestock pests**,** as well as human disease vectors and pests. The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) administers this Act. The USDA had already regulated GM fruit flies and the pink bollworm under the Plant Protection Act (PPA), and completed the first Environmental Impact Statement (EIS) ever done on any LMO, plant or animal, as well as two Environmental Assessments (EAs) on GM insects that are plant pests in accordance with the US National Environmental Policy Act (NEPA). NEPA includes a number of provisions for public stakeholder participation in the Federal decision-making process. There are also litigation precedents in the USA that establish that implementation of FIFRA to be equivalent to NEPA.

Legislation pertaining to mosquito control exists in many countries including Singapore, Malaysia, Tanzania, Australia (Queensland), and the USA (Florida Statutes, 2009). This mainly is for the purpose of enforcing control program requirements, such as elimination of larval habitats by citizens. According to Florida Statutes, the creation, maintenance, or causing of any condition capable of breeding flies, mosquitoes, or other arthropods capable of transmitting diseases, directly or indirectly to humans, are prohibited by regulation. In Malaysia, there are laws for the prevention and control of vector-borne diseases. These are: (a) Destruction of Disease-Bearing Insects Act 1975 (Act 154); (b) Prevention and Control of Infectious Diseases Act 1988 (Act 342); and (c) Local Government Act 1976 (Act 171). The Destruction of Disease-Bearing Insects Act (Seng, 2001) was enforced throughout the country from August 23, 1982. The Destruction of Disease-Bearing Insects (Amendment) Act 2000 came into operation throughout the country on January 1, 2001 (Seng, 2002.) In Singapore, three pieces of legislation, namely, the Infectious Diseases Act (IDA), the Control of Vectors and Pesticides Act (CVPA)

[55] For example, http://www.who.int/rpc/research_ethics/informed_consent/en/

and the Environmental Public Health Act (EPHA) provide broad powers to prevent and control dengue (Seow, 2001). Among those countries in Africa with laws pertaining to breeding of mosquitoes, Tanzanian law goes back to 1913, with legislation governing the breeding of *Anopheles* spp, *Aedes*, spp. and, more recently, *Culex quinquefasciatus*.

GM animals

Genetic modifications to animals are generally for the purpose of affecting their physiology or biology in ways to provide economic or health benefits. Animal drugs or pharmaceuticals also are human interventions intended to similarly affect or alter animal physiology or biology. Legislation for regulation of animal drugs is presently being used to regulate GM animals, including GM salmon, developed for food and drugs. In the USA, the legislation is the Federal Food, Drug, and Cosmetic Act (FFDCA) and the implementing Agency is the Food and Drug Administration (FDA) within the Department of Health and Human Services (HHS). GMM may be regulated in the USA by FDA, Center for Veterinary Medicine (CVM) under FFDCA as animal drugs.

Environmental Protection

Many countries have enacted legislation with regulation by environmental and/or fish and wildlife management agencies for the protection of certain species against adverse effects from human activities. Legislation also exists to protect species that have become threatened or endangered due to human action resulting in potential extinction. Where other regulatory agencies do not have authority because the nature of a LMO may not clearly fit their regulatory scope, environmental agencies may have regulatory purview because of potential adverse impacts on protected species and species diversity in the environment. In this same regard, regulation by other agencies may require endangered and threatened species impact analysis as part of their regulatory process, as is presently required in some countries, including the USA. The Convention of Biological Diversity (CBD, 1992a) and the Cartagena Protocol on Biosafety (CBD, CPB, 2010) are examples of treaties or covenants applying to GMOs/LMOs and are based on protection of species diversity.

Some countries, such as Malaysia, Brazil, Panama, Kenya, and Nigeria, have developed specific legislation for living modified organisms that is based on GM plant experience, but includes other LMOs. Such legislation is usually derived from the CPB, described in Appendix 5.1. New legislation may require a new regulatory agency to be established or draw on other agencies or non-government sources for scientific, regulatory, and enforcement expertise. In countries, where government budgets are limited and capacity building needed, this approach may result in either biotechnology implementation delays or possibly regulatory decisions resulting in risks to humans and the environment, as a result of inadequate science assessment capacity and conflict of interest if regulators are poorly paid and compromised.

Regulation of GMM with drive systems capable of autonomous transboundary movement may invoke regulatory processes of adjacent countries because such gene drive systems are designed and intended to spread throughout an ecozone regardless of political boundaries. If it is known or expected that introduced traits will have transboundary affects, then the need for multilateral regulatory approval by all countries, not separated by species barriers, subject to introduction of a specific GMM should be considered. To engage a multilateral regulatory process may involve international agreements, treaties, covenants, conventions, protocols, or county approvals prior to introduction to one country within a contiguous ecozone. International organizations, such as WHO, may be best suited to provide

leadership in a multilateral/international regional regulatory process for deploying GMM intended to spread widely (see Appendix 5.2 for further discussion).

Table 5.1 Recent regulatory and biosafety development chronology relevant to testing and implementation of modified vector insects

Year	Development	Relevance	Website
2000	Cartagena Protocol on Biosafety to the International Convention on Biological Diversity	Established Biosafety Clearing-House for information on national biosafety regulations and contacts	http://bch.cbd.int/
2001 - 2007	African Model Law on Biosafety	African Union drafted a model legal instrument for developing national biosafety legislations in 2001 that was endorsed by African Ministerial Conference on Science and Technology in November 2007; several African countries have now approved national biosafety laws	http://www.africa-union.org/root/au/auc/departments/hrst/biosafety/AU_Biosafety_2b.htm http://www.africa-union.org/root/au/auc/departments/hrst/biosafety/DOC/level2/African%20Model%20Law%20with%20Annexes-EN.pdf
2002 - 2004	WHO/TDR Technical Consultations on GM Vectors	Began the process of defining requirements for testing and implementation of GM vectors	Alphey *et al* (2002) Science 298:119-121 http://library.wur.nl/frontis/malaria/index.html http://library.wur.nl/frontis/disease_vectors/index.html
2002 - 2007	International Project on LMO Environmental Risk Assessment Methodologies	Identified and developed scientific methodologies and teaching tools that can be used for environmental risk assessment and management of transgenic plants, in accordance with the Cartagena Protocol on Biosafety and other international agreements	http://www.LMOera.umn.edu/
2005	IPPC Guidelines for the Export, Shipment, Import and Release of Biological Control	International Plant Protection Convention approved international standards for risk management related to biological control agents capable of self-replication	https://www.ippc.int/servlet/BinaryDownloaderServlet/76047_ISPM_3_E.pdf?filename=1146657660135_ISPM3.pd

	Agents and Other Beneficial Organisms		f&refID=76047
2006	Daegu Protocol on New Technologies for Pest and Disease Control	International effort initiated to establish guidance for regulation of new biotechnologies related to crop pests and human disease vectors	http://www.biopesticide.ucr.edu/daegu/daegu.html
2006	USDA Environmental Assessment on field release of genetically engineered pink bollworm	USDA Animal and Plant Health Inspection Service announced a final environmental assessment and finding of no significant impact to issue permit for confined field trial in southwest US of transgenic plant pest modified to contain fluorescent marker gene	http://www.scribd.com/doc/2810616/Notice-Environmental-statements-availability-etc-Pink-bollworm-genetically-engineered-to-express-green-fluorescence-as-marker-field-trial
2007	NAPPO Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries	North American Plant Protection Organization approved regional standard to provide guidance in use of transgenic arthropods while protecting plant health	http://www.nappo.org/Standards/NEW/RSPM%20No.%2027-e.pdf
2007	WHO/TDR supported regional centres in Africa, Asia, and Latin America for training in biosafety assessment for human health and the environment of the use of genetically modified disease vectors	Capacity building through a series of training courses targeting researchers, policy makers, regulators, etc. in developing countries for decision making on regulatory frameworks, biosafety, risk assessment, and ESC issues related to use of GM vectors	http://www.tropika.net/svc/funding/20090831-1st-biosafety-training-latin-america http://www.tropika.net/svc/news/20090831/20090831-2nd-lab-biosafety-training-india http://apps.who.int/tdr/svc/grants/calls/3rd-biosafety-training-asia http://apps.who.int/tdr/svc/grants/calls/3rd-biosafety-training-mali
2008 - 2011	MosqGuide project	Development of best practices for the use of GM mosquitoes, to be used as guidance for decision making in disease endemic countries	http://www.mosqguide.org.uk/
2008	FNIH-supported working group on contained field	Development of guidance for the conduct of Phase 2 contained field trials for GM mosquitoes with self-limiting or self-	http://www.liebertonline.com/doi/pdfplus/10.1089/vbz.2007.0273

	trials of vector mosquitoes engineered to contain a gene drive system	sustaining gene drive	
2009	USDA Environmental Impact Statement on Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs	USDA Animal and Plant Health Inspection Service, in cooperation with several States and foreign countries, conducts a full risk assessment of genetically engineered fruit fly species and pink bollworm for use in various applications of the sterile insect technique (RIDL), determines it to be an environmentally preferable alternative to current technology, and announces intention to integrate use of GM insects into agency invasive plant pest control programs	http://cofcs66.aphis.usda.gov/plant_health/ea/downloads/eis-gen-pbw-ff.pdf http://www.epa.gov/fedrgstr/EPA-IMPACT/2009/May/Day-07/i10633.htm
2009	WHO/TDR and FNIH-sponsored technical consultation on progress and prospects for the use of GM mosquitoes to inhibit disease transmission	Reviewed current status and requirements for future development of GM mosquitoes for malaria and dengue control; initiated development of a guidance framework for evaluation of GM mosquitoes including quality standards for assessing safety, efficacy, and ESC considerations (in progress).	http://apps.who.int/tdr/svc/publications/training-guideline-publications/gmm-report
2010	Ad hoc Technical Expert Group on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety	Developed a Roadmap and Guidance on risk assessment and risk management of GMOs to supplement Annex III of the Cartagena Protocol, with a special section on living modified mosquitoes	http://www.cbd.int/doc/meetings/bs/bsrarm-02/official/bsrarm-02-05-en.doc
2010 - 2012	European Food Safety Authority projects relevant to GM insects	Environment Agency Austria, IAEA and the University of Bern produced a scientific/technical report “Defining environmental risk assessment criteria for genetically modified (GM) insects to be placed on the EU market” “Guidelines for GM Animals” (in development)	http://www.efsa.europa.eu/en/scdocs/doc/71e.pdf http://www.efsa.europa.eu/en/gmo/gmowgs.htm
	WHO/TDR	Guidance relating to the requirements for	http://www.mosqguide

	MosqGuide Project, modules 1 through 7	deployment of genetically modified mosquitoes.	.org.uk/]

Regulation in a stepwise research and development process

Regulatory oversight will usually be required in **Phase 1** (See Figure 1.1) for importation and possibly interstate movement permits. Inspections may be conducted to assess the security of quarantine containment according to established guidelines. Institutional biosafety committees, where they exist, would also be involved at the beginning of this stage. Other regulatory requirements could be for permits to rear mosquitoes and for permission to work with human disease vectors and the disease agents, if applicable, in the regulatory jurisdictions where the research is to be conducted. Provisions for surveillance and monitoring for escaped GMM also should be part of the regulatory requirements at Phase 1 because of possible containment failures, since mosquitoes are small and mobile. Regulation at this stage of research and development also should provide for emergency control or mitigation measures to eliminate escaped GMM through proven means, such as pesticide applications. International biotechnology product movement permits and quarantine systems are already established in many countries for movement of living plant and animal agents that may become pests.

Physically and ecologically confined field trials in **Phase 2** should require regulation in which a risk assessment (Section 3: Biosafety) or other similar environmental assessment is conducted and documented to supply scientific rationale and evidence that the confinement will provide the expected degree of assurance that the GMM will not escape into the surrounding environment and become established and spread or result in spread of the genetic construct(s) into native sexually compatible species. Provisions for surveillance and monitoring should also be part of the regulatory requirements at this phase. Regulation should also provide for emergency control or mitigation measures to eliminate escaped and established GMM and constructs through proven methods. Clear distinctions should be made between physically and ecologically confined field trials to define what each means relative to inadvertent dispersion of the GMM because there would likely be different regulatory requirements according to the degree of containment or confinement provided.

Open release trials under **Phase 3** should require regulatory risk assessment or other similar environmental assessment documentation to provide a scientific rationale and evidence that the genetic construct(s) are either self-limiting or self-mitigating, and if not 100% self-limiting, that the releases will not then introduce genetic constructs into indigenous wild populations of vectors that may result in increased biological fitness, increased or broadened disease vector capacity, or increased human and animal nuisance impacts. For constructs that are intended to spread within a vector population for the purposes of population suppression or reducing capacity to transmit diseases, there likewise should be regulatory requirements to establish scientifically that the genetic construct(s) do not otherwise increase biological fitness, broaden vector capacity to other disease agents, or increase human and animal nuisance impacts**.** In case of failure to perform as expected or required, appropriate control or mitigation measures need to be available to eliminate escaped and established GMM. When transboundary movement to adjacent countries or states with separate regulatory jurisdiction is expected or intended, then prior to the release of GMM with genetic constructs capable of expanding in a vector population, the regulatory requirement of the countries or states into which animals containing

the transgene may move also should be addressed (see discussion of Transboundary Movement below). Phase 2 and/or 3 also may require assessment of impact on nontarget and beneficial species and include species that are threatened or endangered in the environment. Satisfactory completion of Phase 3 trials may result in regulatory approvals for programmatic implementation and no longer require regulatory supervision for post implementation when all safety-testing parameters are satisfied.

In **Phase 4**, post implementation surveillance regulation, when required, should be intended, 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 GMM. Where geographical boundaries are not sufficient to prevent transboundary movement to adjacent countries or states, then prior to release of GMM with genetic constructs capable of spreading within a vector population, the regulatory requirement of the countries or states into which the construct may move also should be met.

Additional considerations pertinent to GMM regulation

Burden of excessive risk assessment

Regulatory officials should be aware that funding resources for permits, licenses, or registrations of GMM may be significantly less than for commercial transgenic crops because these insects will be developed most often for the public good by non-profit organizations such as university affiliates, philanthropies or public agencies, or by small biotechnology companies with constrained resources. In consideration of the significant costs that are incurred in the development of risk assessment data, regulatory agencies should be encouraged to focus on science-based, case-by-case targeted requirements with a degree of practical parsimony rather than to rely on a precautionary approach that can require data to address all theoretical risks. Potential benefits to public health should be part of the regulatory risk/benefit analysis.

Conflict of Interest

Most applicable legislation concerning conflict of interest may involve government regulatory officials and agency actions, although conflict of interest also arises when special interest groups lobby and make monetary or other contributions to influence legislation and its passage into law for the benefit of themselves or their sponsors. There may be legislation and enforcement on conflict of interest findings when public funding is used to pay for the process of regulatory decisions with private benefits and when the decision by a government employee or agency is biased by some personal benefit. Conflicts of interest may occur in the regulation of GMM under the following circumstances: 1) regulators who have vested or proprietary interests in the development, approval, and implementation of the biotechnology; 2) regulators who have vested or proprietary interests in preventing the development; 3) regulators who want and gain a professional benefit or other status as a result of approving or disapproving the biotechnology; 4) people, organizations, or companies that are not regulators, but have a vested interest or bias either for or against a biotechnology and have gained a position to influence and advise regulators on approval or disapproval or who may themselves participate directly in the regulatory decision process; and 5) when risk or other environmental assessments are drafted by non-regulatory persons or companies that have bias and may receive payment or other benefit from a regulatory decision based on the assessment, except that third parties without bias may draft such assessments under contract or other agreement with provisions against such bias. Risk assessment reports and data paid for or directed by a license, permit, or registration

applicant are common to many regulatory processes, but provide opportunity for conflict of interest and falsified data when driven by profit motivation. Ways to mitigate conflicts of interest include: 1) removal, so as to avoid them entirely; 2) disclosure of the conflict of interest and recusal of the conflicted party from decisions where the conflict exists; and, 3) third-party evaluations in which the third party has established no conflict of interest, but has expertise and knowledge to make or advise on the decision.

Litigation

Regulation by litigation may occur when the regulation does not have sufficient basis in law, or is flawed by risk assessment that does not meet Good Laboratory Practice and refereed publication standards or by legally required administrative procedures. Litigation or lawsuits, court injunctions, court orders, fines and penalties may then drive the regulatory process, usually after actions have occurred. There have been several such lawsuits over GM/LM crop plants. This is the least desirable regulatory outcome for GMM and may result in the loss or delay of beneficial public health innovation as well as loss of public confidence.

Capacity and institution building as an essential component of an informed regulatory infrastructure

Building of regulatory capacity to evaluate GMM will be unequivocally important. It may be anticipated that there will be a need to train members of national regulatory authorities on issues relevant to the review of entomological intervention trials. In many countries, members of the national regulatory authority will have a pharmacy or medical background with experience in regulating drugs, vaccines, and devices. There is a strong probability that they will be unfamiliar with trials of vector control tools, although there are exceptions (for example, in Tanzania, review of vector control trials is done by the Tropical Pesticides Research Institute).

Moreover, although many developing countries have enacted national biosafety legislation, others still do not have a regulatory framework to deal with GMOs. Even if legislation is present, there may not be a functional system in place to regulate GMM. If experience with risk assessment and regulation of GMOs exists, it is likely that GM plants or crops will provide the only precedent. Because most legislation dealing with GMOs assigns regulatory responsibility to a separate national biosafety authority, and because the focus of those authorities likely will have been on GM crops, the composition of those bodies will consist of members who have little experience with the technologies involved in producing GMM or how to regulate them. Regulatory paradigms set by experience with multinational GM plant or crop corporations may result in prohibitive costs and extended indecision on regulatory approvals. Adoption of a strict interpretation of the precautionary approach or principle (see Appendix 5.2) could mean that regulatory approvals would not be granted until even remote or theoretical risk and safety issues are resolved, regardless of societal needs and potential benefits. This strict interpretation may be incorporated in capacity building efforts conducted by groups opposed to GM technology. Developers of GMMs that have been created for the public good, not for profit, may find it impossible to support excessive risk assessment study costs or sustain operations throughout interminable delays.

Thus, it will be critical to begin working with regulators very early on in a GMM project to identify the appropriate regulatory pathway and to initiate proactive communications that will build understanding about the GMM technology as well as the goals and methodologies of the project. There

may be a need for additional training in vector biology procedures and/or biosafety to ensure that decision makers are empowered to competently assess plans for GMM trials and reach definitive and defensible conclusions, taking into account cost-benefit considerations. These needs must be anticipated, and means to address them must be identified and budgeted for accordingly.

Regulatory precedents for transboundary movement

Transboundary regulatory issues that apply to GMM have been raised because mosquitoes are mobile. For example, the anthropophilic *Aedes aegypti* vector of dengue and other diseases has spread worldwide wherever suitable habitats exist, especially with increasingly favorable peridomestic habitats provided by ever increasing human urbanization. Thus, risk assessment and risk management plans should take into account the possibility that GMM that are not 100% sterile may move autonomously across political borders into suitable habitats that are contiguous, or even into regions separated by geographic or biological barriers due to human travel and transport.

The general consensus of international conventions that address transboundary movement of GMOs or exotic agents, and that therefore may apply to GMM, is that prior to release into the environment or implementation, there should be a notification and a bi- or multilateral consultative process with other countries to which the GMM may spread. With respect to GMM that are disease vectors, this could be within the context of a collaborative process for control of the vector.

Relevant conventions that address transboundary movement include the following:

- The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) (WTO, 1994d), Articles 3, 5, and 6.
- The Convention of Biological Diversity (CBD, 1992a.), Articles 3, 4, 5, 14 and 17.
- The Cartagena Protocol on Biosafety, Articles 4, 6, 8, 14, and 19
- The International Plant Protection Convention (IPPC) Article 7 and IPPC, ISPM, No. 3 and 11 (IPPC, ISPMs. 2009).
- Code of Conduct for the Import and Release of Exotic Biological Control Agents (FAO, 1996).
- The ASEAN Agreement on the Conservation of Nature and Natural Resources, Article 3 (Kuala Lumpur, 1985).
- The Convention of Conservation of Nature in the South Pacific, Article V.
- The Convention for the Conservation of Biodiversity and the Protection of Wilderness Areas in Central America, Article 24.
- The International Health Regulations, as amended, 1982.

A successful example of a multilateral collaborative effort is provided by the introduction the parasitic wasp, *Epidinocarsis lopezi* of the cassava mealybug, *Phenacoccus manihoti,* in Africa (Neuenschwander and Herren, 1988). The parasite was released in more than 50 sites and by the end of 1986, it was established with good results in 16 countries. National introductions were facilitated by inputs from international organizations to guarantee the safety and efficacy of the introductions, including the International Institute of Tropical Agriculture (IITA), the International Institute of Biological Control (IIBC) and the African Union's Phytosanitary Commission (IAPSC).). The IAPSC did not make blanket decisions for member countries and releases were national decisions, once imported into quarantine. The IIBC main concern was to ensure freedom from disease and hyperparasites, while IITA assisted governments with local production, release and monitoring of parasites. IITA also coordinated a

large capacity building element in the program, which helped create a generation of technical people across Africa with knowledge of both biocontrol and quarantine, and this has been helpful to further biocontrol projects in Africa (2011 personal communication from Jeff Waage).).

Another example of a successful regional program is the biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green, in the Caribbean (Kairo et al., 2000).

Appendix 5.1 Examples of National Legislation and Regulation Pertaining to GMM

This appendix provides a brief description of the regulatory framework of several countries that have engaged in or are contemplating GMM research. The most important resource for specific country GMM regulation and contacts is the Cartagena Protocol on Biosafety, Biosafety Clearing-House (CBD, CPB, BCH, 2010). Another source of information is the Convention of Biological Diversity, Biosafety Information Resource Centre (CBD, BIRC, 2010).

Brazil

In Brazil, Federal Law # 11.105[56], of March 2005 is the principal legal framework for biotechnology and provides safety regulation and inspection tools for activities concerning genetically modified organisms and their byproducts. This law was implemented by the National Biosafety Council (CNBS), provided a new format for the National Biosafety Technical Commission (CTNBio), and established a framework through the National Biosafety Policy (PNB). CNBS is linked directly to the Office of the President of Brazil and is responsible for providing the PNB. The CNBS is responsible for establishing principles and guidelines for the administration of federal agencies that regulate biotechnology. Also, CNBS analyses the social-economic impact of commercial use of GMOs and their by-products and issues the final approval of licenses and policies, when deemed necessary.

CTNBio belongs to the Ministry of Science and Technology of the Federal Government of Brazil and is a consulting and deliberating multidisciplinary body that provides technical assistance to support biotechnology decisions at the Federal level. CTNBio is responsible for approvals of research and development of GMOs under specific conditions and approval for tests or commercialization of any biotechnology product for human, animal, and plant use. CTNBio must approve every laboratory or facility that intends to manipulate genes for creation of GMOs prior to operation. The Commission has 27 members that include scientists with biotechnology backgrounds, federal officers, lawyers, and other experts.

In order to have a prior analysis before submission to CTNBio, all organizations (university, research institution, and industry) must have an internal Biosafety Commission that does the initial evaluation of the research. After approval at this first level, the research project is submitted to CTNBio. The requirements for approval of commercial products are quite strict and may take years to be accepted, but mainly involve new plant varieties. After approval, the executing organization must periodically report on implementation and provide results to CTNBio.

Malaysia

The Biosafety Act (2007) (Act 678) established the National Biosafety Board to regulate the release, import, export, and contained use of living modified organisms and the release of their products with the objectives of protecting human, plant and animal health, the environment and biological diversity. The Board consists of the following members: Secretary General of the Ministry of Natural Resources and Environment, who is the Chairman, and representatives from the Ministries of Agriculture and agro-based industry; Ministry of Health, Ministry of Plantation Industries and Commodities; Ministry of Domestic Trade and Consumer Affairs; Ministry of International Trade and Industry; Ministry of Science, Technology, and Innovation; and not more than four other persons who have the knowledge or experience or both in any of the disciplines or matters relevant to this Act. A

[56] Brazil. 2005. Lei No 11.105, de 24 de Marco de 2005. Presidencia da Republica, Brasilia, Brazil. http://www.planalto.gov.br/ccivil_03/_Ato2004-2006/2005/Lei/L11105.htm

Director General shall be the Secretary of the Board to carry out such duties as may be required by the Board.

The stated functions of the Board are to: decide on all applications; to monitor activities relating to living modified organisms and products of such organisms; promote research, development, education and training activities relating to biosafety; and establish mechanisms to facilitate the collection, storage and dissemination of data relating to living modified organisms and products of such organisms and biosafety. A committee by the name of the "Genetic Modification Advisory Committee" is established to provide scientific, technical and other relevant advice to the Director General.

An application for the approval of any release activity, or any importation of living modified organisms, or both shall be submitted to the Director General and shall be accompanied with a risk assessment, a risk management report, and an emergency response plan. The risk assessment and risk management reports shall be in a form prescribed by the Minister and shall contain an assessment of the risk and adverse effect that such living modified organisms and products of such organisms will have or are likely to have on human, plant, and animal health; the environment and biological diversity; and the proposed measures that shall be undertaken to prevent, reduce or control the risks and adverse effects that such living modified organisms and products of such organisms will have or are likely to have. The emergency response plan shall provide safety measures and procedures for the protection of human, plant, and animal health, the environment, and biological diversity against harm or damage caused directly or indirectly by living modified organisms or products of such organisms, and all necessary measures to be taken in the event of an emergency.

Information on Malaysian biosafety regulations and the National Safety Board decision to approve GMM experimentation can be obtained from www.biosafety.nre.gov.my.

Mexico

Mexico actively participated in negotiations leading to the Agreement on Biological Diversity and when the Cartagena Protocol on Biosecurity was adopted. The Interministerial Commission on Biosecurity and Genetically Modified Organisms (CIBIOGEM, http://www.cibiogem.gob.mx/Acerca/Paginas/default.aspx) was created by Presidential Decree on the 5 November 1999 (Villalobos, 2006.) Under Mexican Federal law, CIBIOGEM functions to: present suggestions to the National Normalization Commission about Mexican official norms for the research, production, trade, import, export, movement, commercial use, and consumption of LMOs; promote, together with CONABIO (Comisión Nacional para el Uso y Conocimiento de la Biodiversidad, National Commission on the Use and Knowledge of Biodiversity) the establishment of a data bank on the presence and distribution of native species related to LMOs, and monitor mechanisms and evaluate the environmental impact and the impact on human and animal health resulting from the production and consumption of LMOs; set up a uniform program for the inspection of LMO research and production plants; and, recommend methods for the dissemination of information regarding the benefits and possible risks of the use and consumption of LMOs to the public.

Additionally, the 1999 decree established the Executive Secretary, the Technical Committee, and the Consultative Council on Biosecurity. The Executive Secretary responsibilities include, but are not limited to: ensuring that laws regarding biosecurity and the regulations of CIBIOGEM are followed by government institutions; registering LMOs and their products and sub-products; establishing and maintaining an up-to-date registry of LMOs; and, establishing and maintaining an up-to-date data bank regarding the presence and distribution of native species related to LMOs. The activities of the Technical Committee are coordinated by the Executive Secretary of CIBIOGEM, and include preparing and suggesting to the Executive Secretary issues and regulations that have to be submitted for consideration by CIBIOGEM; and, when suggested by CONABIO, reaching agreements with the

responsible institutions regarding the performance of risk analyses for LMOs and their products and sub-products.

USA

The USA is not a signatory agent to the Cartagena Protocol on Biosafety (CPB) and uses its existing National legislation and agencies to regulate LMOs under the Coordinated Framework for Regulation of Biotechnology, (USA, OSTP, 1986). The June 26, 1986 Coordinated Framework for Regulation of Biotechnology exists as an Executive Office of the President, Office of Science and Technology Policy Federal Register 51 FR 23302 announcement of policy notice for public comment, and is a guidance and not a law in the USA.

In summary, this Federal Register notice announces the policy of the Federal agencies involved with the review of biotechnology research and products. This notice includes separate descriptions of the regulatory policies of FDA, EPA, OSHA, and USDA and the research policies of the National Institutes of Health (NIH), National Science Foundation (NSF), EPA, and USDA. The agencies will seek to operate their programs in an integrated and coordinated fashion and together should cover the full range of plants, animals, and microorganisms derived by the new genetic engineering techniques. To the extent possible, responsibility for a product use will lie with a single agency. Where regulatory oversight or review for a particular product is to be performed by more than one agency, the policy establishes a lead agency and consolidated or coordinated reviews.

While in part certain USDA and EPA requirements are new, the underlying regulatory regimens are not new. Members of the agricultural and industrial communities are familiar with the general requirements under these laws, which include the Federal Plant Pest Act, The Plant Quarantine Act, the Toxic Substances Control Act (TSCA), Federal Food, Drug, and Cosmetic Act (FFDCA), and the Federal insecticide, Fungicide, and Rodenticide Act (FIFRA). Because this comprehensive regulatory framework uses a mosaic of existing Federal law, some of the statutory nomenclature for certain actions may seem inconsistent. Certain laws, such as USDA's Federal Plant Pest Act, require a "permit" before a microorganism pathogenic to plants may be transported or imported. Under other laws such as FIFRA, the agencies “license” or “approve” the use of particular products. TSCA requires a “premanufacturing notification (PMN)”. There are also some variations among the agencies in the use of the phrase “genetic engineering.” Agencies have agreed to have scientists from each other's staff participate in reviews. Each regulatory review will require that the safety, or safety and efficacy, of a particular agricultural or industrial product be satisfactorily demonstrated to the regulatory agency prior to commercialization.

The National Environmental Policy Act (NEPA) imposes procedural requirements on all Federal agencies to prepare an analysis prior to making a decision to take any action that may significantly affect the environment. Depending on the characteristics of a proposal, an environmental assessment (EA), or a broader environmental impact statement (EIS) may need to be prepared in connection with the release of genetically manipulated organisms. EPA's actions under most of its environmental statutes have been considered to be the functional equivalent of NEPA compliance.

Specifically relevant to GM insects, USDA, APHIS has Transgenic Insect Permit Guidance publicly available (USDA, APHIS, 2007). Threatened and endangered species impact assessment is required under the Endangered Species Act (ESA). Federal regulatory decisions regarding permits for GMO environmental release in the USA are subject to either EA for some trials or EIS for large-scale or programmatic use under NEPA. The EPA pesticide/biopesticide registration process has been determined by court decision precedent to be legally equivalent to NEPA.

Two EAs and one programmatic Final EIS on Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs (USDA, APHIS, 2008), have been conducted for use of GM Mediterranean, Mexican, and Oriental fruit flies and the pink bollworm of cotton. The two EAs and

the EIS included environmental risk assessments (Rose, 2009). The EIS was both a USA and international precedent because it was the first EIS ever done on any LMO in the USA or elsewhere under comparable environmental laws of other countries. EA and/or EIS environmental documentation required in the Federal decision making process must provide for alternatives so that different approaches may be considered besides the preferred or proposed alternative. The Record of Decision for the final EIS on Use of Genetically Engineered Fruit Flies and Pink Bollworm authorized the development and use of these genetically engineered insects in sterile insect technique (SIT) for USDA/state cooperative plant pest eradication and control programs. The two EAs and the EIS were published for public comment in the US Federal Register and many comments were received and published, which were then evaluated in the decision-making process and final public documents. Field release testing of genetically modified pink bollworm has been conducted in the USA.

European Union

In the European Union (EU), a formal risk assessment is the mechanism by which the risks of the release of a LMO are evaluated. The benefits of such a release are not taken into account within a risk assessment in the EU. The release of a GM insect within any EU member state is controlled by a directive of the European Parliament and of the Council, known as the Deliberate Release Directive (EU Directive 18/EC, 2001), which regulates release of all LMOs into the environment. For example, the release of a GM insect in the UK is controlled by the Deliberate Release Directive. In the case of a non-commercial release, such as a field trial, the decision to approve release would be made at the UK National level by the Department for Environment, Food, and Rural Affairs (DEFRA) in consultation with the independent scientific experts of its Advisory Committee on Releases to the Environment (ACRE), which is responsible for assessing the risks of the technology. For a commercial release, DEFRA would perform an initial evaluation of the application with ACRE's input. This application would then be sent to every EU member state with the European Food Safety Authority (EFSA) providing a scientific opinion. Member states must then reach a qualified majority to approve any release based on scientific evidence. Should the member states fail to reach a decision, the application then passes to the European Commission, which can approve or deny the application based on the scientific opinion of EFSA. The EFSA is currently developing a Guidance Document on the Environmental Risk Assessment (ERA) of Genetically Modified Animals, including insects.

Appendix 5.2 Guidance to Additional Information Relevant to GMM Regulation

International Organizations, Treaties and Covenants

The **World Trade Organization (WTO)** Agreements and Public Health; A Joint Study by WHO and the WTO Secretariat (WHO/WTO, 2002). This study explains how WTO Agreements relate to different aspects of health policies. It covers several areas including infectious disease control, environment, and biotechnology. The study explains that countries have the right to take measures to restrict imports or exports of products when necessary to protect the health of humans, animals, or plants. If necessary, governments may put aside WTO commitments in order to protect human life. The study discusses application of biotechnology to foods and potential health effects such as gene transfer from plants to microbial or mammalian cells, transfer of antibiotic resistance, and allergenic effects.

The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (**SPS Agreement**) (WTO, 1994d) articles include, but are not limited to the following, which may pertain to autonomous transboundary movement of GMM:

> Article 1, General provisions - This Agreement applies to all sanitary and phytosanitary measures, which may, directly or indirectly, affect international trade. A sanitary or phytosanitary measure is any measure applied to protect animal or plant life or health within the territory of a member from risks arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms, or disease-causing organisms.
>
> Article 2, Basic rights and obligations - Members have the right to take sanitary and phytosanitary measures necessary for the protection of human, animal or plant life and health.
>
> Article 3, Harmonization - To harmonize sanitary and phytosanitary measures on as wide a basis as possible, members shall base their sanitary or phytosanitary measures on international standards, guidelines, or recommendations. Members shall play a full part, within the limits of their resources, in the relevant international organizations and their subsidiary bodies, in particular the Codex Alimentarius Commission, the International Office of Epizootics, and the international and regional organizations operating within the framework of the International Plant Protection Convention, to promote the development and periodic review of standards, guidelines, and recommendations with respect to all aspects of sanitary and phytosanitary measures.
>
> Article 5, Assessment of Risk and Determination of the Appropriate Level of Sanitary or Phytosanitary Protection - Members shall ensure that their sanitary or phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to human, animal, or plant life and health, taking into account risk assessment techniques developed by the relevant international organizations. In the assessment of risks, members shall take into account available scientific evidence; relevant processes and production methods; relevant inspection, sampling and testing methods; prevalence of specific diseases or pests; existence of pest- or disease-free areas; relevant ecological and environmental conditions; and quarantine or other treatment.
>
> Article 6, Adaptation to Regional Conditions, Including Pest- or Disease-Free Areas and Areas of Low Pest or Disease Prevalence - Members shall ensure that their sanitary or phytosanitary measures are adapted to the sanitary or phytosanitary characteristics of the area, whether all of a country, part of a country, or all or parts of several countries from which the product originated and to which the product is destined.

> Article 12, Administration - A Committee on Sanitary and Phytosanitary Measures is hereby established to provide a regular forum for consultations. It shall carry out the functions necessary to implement the provisions of this Agreement and the furtherance of its objectives, in particular with respect to harmonization.

The SPS Agreement, Module 8.1, Genetically Modified Organisms (LMOs) (WTO, 1994e) recognizes standards developed by the International Plant Protection Convention and the World Organization for Animal Health and applies them to LMOs in respect to the following:

- Protection of human or animal life from risks arising from additives, contaminants, toxins, or disease-causing organisms in food, beverages, and feedstuffs;
- Protection of human life from plant- or animal-carried diseases (zoonoses);
- Protection of animal or plant life from pests, diseases, or disease-causing organisms and;
- Protection of a country from damage caused by the entry, establishment, or spread of pests.

Regulations on GMM should conform to the provisions of this Agreement, such as scientific risk assessment and least trade-restrictive measures.

The WTO Agreement on **Technical Barriers to Trade** (**TBT**) (WTO, 1994f) allows governments to take appropriate measures if they have a legitimate objective, such as protecting health or the environment.

The Convention of Biological Diversity (CBD, 1992a.) Since the adoption of the Convention, the Conference of the parties have initiated national action plans in over 100 countries and raised biodiversity awareness, which led to the adoption of the **Cartagena Protocol on Biosafety** (CBD, CPB, 2010). Mechanisms for implementing the CBD consist of National Biodiversity Strategies and Action Plans (NBSAPs). The articles of the CBD that may pertain to GMM transboundary movement include the following:

> Article 3, Principle - States have the sovereign right to exploit their own resources pursuant to their own environmental policies and the responsibility to ensure that activities within their jurisdiction do not cause damage to the environment of other states or of areas beyond the limits of national jurisdiction.
>
> Article 4, Jurisdictional Scope - The Convention applies to each contracting party, regardless of whether the effects of their activities occur within or beyond the area of their national jurisdiction.
>
> Article 5, Cooperation - Each party shall, as far as possible and as appropriate, cooperate with other contracting parties, directly or through competent international organizations in respect of areas beyond national jurisdiction.
>
> Article 8, In-situ Conservation - Each party shall establish or maintain means to regulate, manage, or control the risks associated with the use and release of living modified organisms, which are likely to have adverse environmental impacts, taking into account the risks to human health.
>
> Article 14, Impact Assessment and Minimizing Adverse Impacts - Each Party shall introduce appropriate procedures requiring environmental impact assessment of its proposed projects that are likely to have significant adverse effects and allow for public participation. Each party shall promote, on the basis of reciprocity, notification, exchange of information, and consultation; bilateral, regional, or multilateral arrangements within the area under jurisdiction of other states. Each Party shall notify immediately affected states of danger or damage.
>
> Article 17, Exchange of Information - The contracting parties shall facilitate the exchange of information from all publicly available sources relevant to the conservation and sustainable use of biological diversity, taking into account the special needs of developing countries.

The **Cartagena Protocol on Biosafety** (**CPB**) is the most significant internationally ratified treaty to influence regulation of GMM in developing countries. It is a supplementary agreement to the CBD and is an international treaty governing the movements of living modified organisms (LMOs). It entered into force September 2003 when the number of signatory-joining countries reached 50 and it now includes at least 160 nations, including most developing countries. The CPB affirms the precautionary approach contained in Principle 15 of the **Rio Declaration on Environment and Development** and **Annex II** of the **Deliberate Release Directive of the European Economic Community** requiring regulators to consider all potential risks, even when there is scientific uncertainty about their extent or existence. Principle 15 of the Declaration states the following: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (CBD. 1992b).

The precautionary principle or approach is analyzed in the published Commission of the European Communities Communication on the Precautionary Principle; see (http://ec.europa.eu/dgs/health_consumer/library/pub/pub07_en.pdf).
European Union codifications of the precautionary principle are further described in the Summaries of EU legislation at: http://europa.eu/legislation_summaries/consumers/consumer_safety/l32042_en.htm
In the precautionary principle or approach, if an action or policy has a suspected risk of causing harm to the public or to the environment, in the absence of scientific consensus that the action or policy is harmful, the burden of proof that it is not harmful falls on those taking the action. This principle allows policy makers to make discretionary decisions in situations where there is the possibility of harm from taking a particular course or making a certain decision when extensive scientific knowledge on the matter is lacking. The principle implies that there is a social responsibility to protect the public from exposure to harm when scientific investigation has found a plausible risk, but interpretation has been extended by some to mean that regulatory approvals should not be granted until all possible or theoretical risk and safety issues are scientifically resolved, regardless of societal needs and potential benefits.

A significant provision of Protocol Article 21 is the establishment of the Biosafety Clearing-House (BCH) (CBD, CPB, BCH, 2010) for the compilation and international exchange of important information on movement and release of genetically modified organisms. This useful database contains information relevant to LMOs and national legislation with some governments having provided their biosafety regulatory frameworks and other pertinent regulatory information including important contacts. The BCH purpose is to (a) facilitate the exchange of scientific, technical, environmental, and legal information on, and experience with living modified organisms; and (b) assist parties to implement the CPB.

The Biosafety Information Resource Centre (BIRC) (CBD, BIRC, 2010) is an electronic catalogues of biosafety-related publications and information resources including: news services, e-mail list servers, online databases and search engines, reports and case studies, journals, newsletters, and teaching materials (manuals, toolkits, and presentations). Its objective is to increase the accessibility and utilization of available biosafety information and resources for policymakers, educators, researchers, and the general public.

Whereas national regulations take precedence, aspects of the CPB to be considered for planning of field trials of GE mosquitoes are the following:

> Protocol Article 4 - The Protocol applies to the transboundary movement, transit, handling, and use of LMOs, taking also into account risks to human health. Under the protocol, a country that

wants to export LMOs for intentional introduction into the environment must seek advance informed agreement from the importing recipient country.

Article 6 - The provisions of this Protocol with respect to the advance informed agreement procedure shall not apply to LMOs in transit and transboundary movement of LMOs destined for contained use. Contained use means any operation, undertaken within a facility, installation, or other physical structure, which involves LMOs that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.

Article 8 - Pertains to notification and that "The notification shall contain, at a minimum, the information specified in Annex I."

Article 10 **-** Concerns decision procedures and that decisions taken by the party of import shall be in accordance with Article 15, which addresses risk assessment.

Article 14 - Concerns bilateral, regional and multilateral agreements and arrangements. "The Parties shall inform each other, through the Biosafety Clearing-House, of any such bilateral, regional and multilateral agreements and arrangements that they have entered into."

Article 19 - Regarding competent national authorities, states "Each Party shall designate one or more competent national authorities, which shall be responsible for performing the administrative functions required by this Protocol and which shall be authorized to act on its behalf with respect to those functions."

Articles 8, 10, and 13 and Annex III Concerns environmental risk assessment, taking into account human health.

Part II of the Final Report of the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management under the CPB (CBD, CPB, AHTEG, 2010) on Specific Types of LMOs and Traits, C. Risk Assessment of Living Modified Mosquitoes addresses the following:

- Scope: This document focuses on the specific aspects of risk assessment of LM mosquitoes developed to be used in the control of human and zoonotic diseases.
- Issues to be considered in the risk assessment:
    - effects on biological diversity (species, habitats, and ecosystems);
    - new or more vigorous pests, especially those that have adverse effects on human health;
    - harm to or loss of other species; and
    - disruption of ecological communities and ecosystem processes.
- Gene flow:
    - gene flow through cross-fertilisation;
    - horizontal gene flow; and
    - persistence of the transgene in the environment.
- Evolutionary responses (especially in target mosquito vectors or pathogens of humans and animals) and
- Risk management strategies.

In February 1999, the African Group in the CBD and the Organization for African Unity (OAU, now the AU) began to develop the African Model Law on Safety in Biotechnology. Its first purpose was to provide for a harmonized approach towards biosafety in Africa serving as a model legal instrument for developing national biosafety legislations (AU, 2008).

The **International Plant Protection Convention (IPPC),** Living Modified Organisms and Pest Risk Analysis (Devorshak, C. 2006) discussed the following of relevance to transboundary movement of LMOs. The International Plant Protection Convention (IPPC) is a multilateral treaty with the purpose of protecting plants and plant health from the introduction and spread of pests of plants and to promote measures for the control of plant pests. Biological control agents used to control plant pests fall under the scope of the IPPC. The IPPC is identified in the World Trade Organization's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) as the international standard setting organization for plant health and both the IPPC and SPS Agreement also affirm the sovereign right of all members nations to take necessary measures to protect plant life or health from the introduction and spread of pests. Members of the WTO are legally obligated to base their phytosanitary measures on international standards for phytosanitary measures (ISPMs) developed under the auspices of the IPPC. Like the SPS Agreement and the IPPC, the CPB also requires countries to base measures for LMOs on risk assessment. An open-ended expert Working Group that met in June 2000 that included phytosanitary experts and representatives of the CBD agreed that organisms that do not pose a threat to plant health (e.g. transgenic mosquitoes) do not fall within the scope of the IPPC.

Provisions of the IPPC that may be relevant to GMM research and implementation include the following:

IPPC Standards for Phytosanitary Measures (IPPC, ISPMs, 2009) - Contain guidance that may be useful for adopting and incorporating into national regulation of GMM, especially pertaining to international movement, release, and risk assessment.

IPPC ISPM No. 2, Framework for Pest Risk Analysis (IPPC, ISPMs 2009) - This standard provides a framework that desc ribes the pest risk analysis (PRA) process within the scope of the IPPC. It introduces the three stages of pest risk analysis: initiation, pest risk assessment, and pest risk management.

IPPC Guidelines for the Export, Shipment, Import, and Release of Biological Control Agents and Other Beneficial Organisms ((ISPM No. 03 (2005)) - This standard provides guidelines for risk management related to the export, shipment, import, and release of biological control agents and other beneficial organisms. It lists the related responsibilities of contracting parties to the IPPC, national plant protection organizations (NPPOs) or other responsible authorities, importers, and exporters. The standard addresses biological control agents capable of self-replication (including predators, parasites, nematodes, phytophagous organisms, and pathogens, such as fungi, bacteria, and viruses, as well as sterile insects and other beneficial organisms and also includes those packaged or formulated as commercial products. Provisions are also included for import for research in quarantine facilities of non-indigenous biological control agents and other beneficial organisms. The scope of this standard does not include living modified organisms.

The IPPC includes the following provision in relation to the regulation of biological control agents and other beneficial organisms. Article 7(1) states: "With the aim of preventing the introduction and/or spread of regulated pests into their territories, contracting parties shall have sovereign authority to regulate, in accordance with applicable international agreements, the entry of plants and plant products and other regulated articles and to this end, may...c) prohibit or restrict the movement of regulated pests into their territories and d) prohibit or restrict the movement of biological control agents and other organisms of phytosanitary concern claimed to be beneficial into their territories."

Contracting parties (member nations) should designate an authority with appropriate competencies to be responsible for export certification and to regulate the import or release of biological control agents and other beneficial organisms. The responsible authority should:

- carry out pest risk analysis prior to import or release of biological control agents and other beneficial organisms;

> ● ensure, when certifying exports, that the regulations of importing countries are complied with;
> ● provide and assess documentation as appropriate, relevant to the export, shipment, import or release of biological control agents and other beneficial organisms;
> ● ensure that biological control agents and other beneficial organisms are taken either directly to designated quarantine facilities or, if appropriate, passed to mass-rearing facilities or directly released into the environment;
> ● ensure that importers and, where appropriate, exporters meet their responsibilities; and
> ● consider possible impacts on the environment, such as impacts on non-target invertebrates.

IPPC, ISPM, No. 11, Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms (IPPC, ISPMs 2009) - This standard provides details for the conduct of pest risk analysis (PRA) to determine if pests are quarantine pests. It describes the integrated processes to be used for risk assessment, as well as the selection of risk management options.

S1 includes details regarding the analysis of risks of plant pests to the environment and biological diversity, including those risks affecting uncultivated/unmanaged plants, wild flora, habitats, and ecosystems contained in the PRA area.

S2 includes guidance on evaluating potential phytosanitary risks to plants and plant products posed by living modified organisms (LMOs).

Food and Agriculture Organization, Code of Conduct for the Import and Release of Exotic Biological Control Agents (FAO, 1996.) The objectives of this Code are to facilitate the safe import, export and release of exotic biological control agents by introducing internationally acceptable procedures for all public and private entities involved particularly where national legislation to regulate their use does not exist or is inadequate. The Code describes the shared responsibility of the many segments of society involved and the need for cooperation between importing and exporting countries. Standards are described that encourage responsible and generally accepted trade practices, assist countries to design regulations to control the suitability and quality of imported exotic biological control agents; and to address the safe handling, assessment, and use of such products. Responsibilities are outlined for the entities which are addressed by this Code, including governments, individually or in regional groupings; international organizations; research institutes; industry, including producers, trade associations, and distributors; users; and public-sector organizations such as environmental groups, consumer groups, and trade unions. All references in this Code to a government or governments shall be deemed to apply equally to regional groupings of governments for matters falling within their areas of competence. Governments should designate the competent authority empowered to regulate or otherwise control and, where appropriate, issue permits for the importation and release of biological control agents. If the organism has already been imported and is currently being held in containment, or if the organism is being imported directly for release, the organization should prepare a dossier for submission to the national authority. It should include among other information, a risk assessment to estimate the possible environmental impact in the new area in which any possible risks to animal and human health should be identified. This authority should consult with authorities in neighboring countries within the same ecological area and with relevant regional organizations to clarify and resolve any potential conflicts of interest that may arise between countries. Where problems (i.e. unexpected deleterious incidents) are identified, the authority is to consider, and where appropriate, ensure corrective action is taken and should inform all relevant interested parties.

The **North American Plant Protection Organization**, NAPPO, RSPM No. 27, Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries (NAPPO,

RSPM 27, 2007) is a standard designed to provide guidance to NAPPO member countries (Canada, Mexico, and the USA) on importation and confined field release of transgenic arthropods that are known plant pests or have the potential to affect plant health. This includes transgenic arthropods used for biological control and transgenic beneficial arthropods with the potential to affect plant health. Transgenic arthropod species that are not plant pests, but that may pose a phytosanitary risk, because of genetic modification may also be considered under this standard. Issues relating to the potential adverse impact of transgenic arthropods on human and animal health or on biological diversity and the environment beyond direct and indirect impacts on plant health are not relevant to plant pest issues and fall outside the scope of this NAPPO Standard. Guidance for unconfined release of transgenic arthropods into the environment is not provided in this Standard.

The **International Organization for Biological Control** is an international body involved with transgenic organisms. It has set up a global Working Group on LMO's in integrated plant production (IOBC, 2003).

The **World Organization for Animal Health** (OIE, 2010) was founded in 1924 and is the world organization for animal health. Some standards developed by the OIE deal with diseases that have human health and biosafety significance. The OIE has had a working group on biotechnology since 1996. The OIE is principally concerned with animal or livestock health issues that may be associated with GM animals and vaccines. Examples of subjects from OIE sources involving biotechnology include:

- regulations governing veterinary medicinal products containing genetically modified organisms in the European Community,
- biotechnology applications in animal health and production,
- disease-resistant genetically modified animals,
- DNA vaccines for aquaculture, and
- traceability of biotech-derived animals.

Reports, Studies and Initiatives

The **Report on Defining Environment Risk Assessment Criteria for Genetically Modified Insects to be Placed on the EU Market** (Benedict*, et al*., 2010), written by the Environment Agency Austria, International Atomic Energy Agency and the University of Bern, describes the ongoing developments in the field of GM-arthropods (transformed species, development purposes, and construction of GM-arthropods), and identifies potential adverse effects, as well as methods to investigate them. Crucial arthropod characteristics and necessary baseline information are discussed and the surrogate and modeling approaches evaluated for utility regarding the environmental risk assessment (ERA) of GM-arthropods…It was concluded that "the ERA of GM-arthropods should consider various issues regarding the genetic modification, the respective species, and the receiving environment. Potential risks could be identified concerning gene flow and its consequences, effects on target and non-target organisms, management practices and measures, biogeochemical processes and human health. Since potential risks depend on the method used for modification, the purpose of the GM-arthropod and the species itself, it is recommended to follow a case-by-case approach for the ERA of GM-arthropods."

The **International Project on LMO Environmental Risk Assessment Methodologies Project** (IPLMO, 2010) is an initiative driven by public sector scientists, most who have strong expertise in environmental science, as well as biotechnology, and socioeconomics. The project has identified and developed scientific methodologies and teaching tools (LMO, ERA Project, 2008) that can be used for

environmental risk assessment (ERA) and management of transgenic plants in accordance with the Cartagena Protocol on Biosafety and other international agreements. IPLMO has also produced a Problem Formulation and Options Assessment Handbook (PFOA), which is a guide to the PFOA process and how to integrate it into an ERA of LMOs (LMO, ERA Project, 2007). The PFOA relies upon being transparent, inclusive of all appropriate stakeholders, and rationally informed by the best available science.

The **MosqGuide** project (www.mosqguide.org.uk/) is funded by the World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases (TDR) to provide best practice guidance for deployment of GMM to control mosquito-borne disease. The project is developing a series of modules dealing with: 1) overview of technology options, social, and regulatory issues; 2) technology research and production phase decisions; 3) pre-deployment country decisions; 4) data handling and environmental monitoring; 5) field survey on attitudes for alternative control methods; 6) curricula for capacity building; and 7) a prototype issues and response model.

The **Daegu Protocol** (2007) proposes regulatory use of an Environmental Impact Assessment (EIA), which may also be known as a Strategic Environmental Assessment or Environmental Impact Statement, according to the country of use, but the purpose and content are generally similar. The guidance is based on the use of EIA documentation and analysis, commonly used in North America, the European Union, Canada, Australia, and other countries as a format to provide for public transparency of the process and to meet country government regulatory agency decision-making requirements. The EIA is a document that is developed openly to the public with all available scientific, societal, and stakeholder input. Therefore, the public is provided opportunity to be informed and comment on decisions to release new forms of biotechnology into the environment before release occurs.

Publications and Conference Reports

The objective of a meeting on status and risk assessment of the use of transgenic arthropods in plant protection, proceedings of a technical meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and the Secretariat of the International Plant Protection Convention (Devorshak, 2006) were to: (1) review the current state of the art of transgenic insect technology; (2) review the current regulatory framework in different countries; and (3) develop a set of guidelines for risk assessment of transgenic insects. Presentations addressed regulatory issues in Zimbabwe, Mexico, Argentina, New Zealand, and the USA. The participants concluded that regulatory approval of any transgenic arthropod release will be on this case-by-case basis. With transgenic technology, Intellectual Property Rights of the strains will need to be addressed and the commercial deployment of a transgenic strain will require a complex set of negotiations related to licensing and royalty payments.

From a **Risk Assessment Workshop on Transgenic Insects** held in Kuala Lumpur, November 2008, sponsored by the United Nations Development Programme (UNDP) (Beech et al. 2009), a published paper on Deployment of Innovative Genetic Vector Control Strategies: "Progress on Regulatory and Biosafety Aspects, Capacity Building and Development of Best-Practice Guidance" reviewed current regulation of GMM in respect to the Cartagena Protocol on Biosafety and individual country needs.

The report from "Progress and Prospects for the use of Genetically Modified Mosquitoes to Inhibit Disease Transmission: Technical Consultation on Current Status and Planning for Future Development of Genetically Modified Mosquitoes for Malaria and Dengue Control" (WHO, TDR, 2010) includes

presentations on GMM mosquito technologies in development; community communication and collaboration strategies; public engagement; ecological risk assessment; the transgenic insect Environmental Impact Statement done in the USA; Malaysia's GMM regulatory experience; ethical, legal and social implications; and guidance on GMM testing and development. Regulatory discussions included the trilateral North American Plant Protection Organization standard, ratified in October 2007, the guidance developed by US Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) for permits; and the International Plant Protection Convention (IPPC) standard on deployment of beneficial organisms.

"Guidance for Contained Field Trials of Vector Mosquitoes Engineered to Contain a Gene Drive System: Recommendations of a Scientific Working Group" (Benedict et al., 2008). Section 8, concerns regulation of Genetically Engineered (GE) Mosquitoes and the following topics were addressed in this section: 1) regulation at different international and national levels; 2) regulatory costs; 3) regulatory impact; 4) international organizations and covenants with potential relevance to genetically engineered vector mosquitoes, including the Cartagena Protocol on Biosafety; 5) addressing regulatory requirements; 6) a proactive approach to regulatory approval; and 7) the USDA, APHIS Environmental Impact Statement on GM insects.

"Ethical, Social, and Cultural Considerations for Site Selection for Research With Genetically Modified Mosquitoes" (Lavery et al., 2008) addresses regulatory issues and administrative discussions and concluded the following: "The prevailing international framework governing the import of GM organisms is the Cartagena Protocol on Bio-safety…Signatories of the Cartagena Protocol (and countries that voluntarily acceded to the terms of the agreement without being formal signatories) are required to establish mechanisms to deal with the import and regulation of GM organisms…The process of determining the key authorities proved to be extremely important, because it provided a clear point of contact (in at least one candidate country) to address detailed questions related to the proposed research…Because all research activities must conform to local laws, it is important to have a clear understanding of what laws deal with the issues in the host country, especially if specific legislation is not yet in force…It is common, under these conditions, for activities related to the import and research with LMOs to be conducted under the auspices of a battery of existing laws, each of which might address specific elements of the proposed import and research uses…Another regulatory issue with important implications for the ethics of research involving GM insects is the requirement for risk assessment before the research, which varies from country to country…This issue may be particularly contentious with respect to environmental impact assessments of the research, which may be a regulatory requirement…and thus may be a formal requirement for the investigators."

A monograph on "Ethical, Legal and Social Issues of Genetically Modifying Insect Vectors for Public Health" (Macer, 2005) considered a range of ethical issues including animal rights, informed consent, community consensus and environmental viewpoints and states that each community needs to decide its own priorities for methodology of disease policy guidance for ethical genetic engineering and to negotiate with neighboring countries. "The approach to genetically modify insects raises few intrinsic ethical issues; however, important environmental and human health concerns need to be assessed before release of any GM insects…The policy that each community adopts should be the product of open dialogue involving all sectors of society. It can be expected that this process will take years and not all communities will endorse genetic control approaches to insect vectors."

An article entitled "When Biotech Crosses Borders" (Angulo and Gilna, 2008) states that rapid action is needed to address loopholes in the international governance of self-dispersing genetically modified organisms (LMOs) purposefully released for the management of wild species and diseases.

A letter to the editor in Nature Biotechnology by John M. Marshall (2010) titled "The Cartagena Protocol and Genetically Modified Mosquitoes" discussed Part II, C. Risk Assessment of Living Modified Mosquitoes, and poses issues and a call for a broader discussion on GM mosquitoes to address their unresolved biosafety concerns. The author proposed that "Perhaps the most important issue inadequately addressed by the guidance document is the ability of mosquitoes engineered with gene drive systems to propagate transgenes across national borders in the absence of an international agreement…The scenario of containment is particularly relevant to GM mosquitoes because, before an open release, trials are being discussed that would take place in field cages exposed to the ambient environment in a location that the species naturally inhabits."

Another article on "Biosafety Concerns Involving Genetically Modified Mosquitoes to Combat Malaria and Dengue in Developing Countries" was recently published by Ostero and Gostin (2011). This article considered elimination of the 3,500 global species of mosquitoes as extraordinarily complex, proposed a new global treaty on genetic or biological modification of vectors, and suggested that modified arthropods should be released only as a last resort. The article was rebutted in a letter to the editor on "Safety of Genetically Modified Mosquitoes" by Benedict et al. (2011), which noted that only a very few species (those serving as vectors for malaria and dengue) are the subject of genetic engineering out of the 3,500 known species and pointed out the presently existing adverse environmental impacts of pesticide use and other methods, including draining wetland mosquito habitats, currently employed for mosquito control.

"A Guide to Designing Legal and Institutional Frameworks on Alien Invasive Species" (Shine, C. et al., 2000) is a chapter that addresses alien species including those that may be unintentionally introduced and LMOs as a subset of alien species stating that: "it is possible that the release or escape of transgenic, recombinant or novel DNA might have severe and irreversible effects on environmental safety." Potential health impacts are discussed in respect to invasive microorganisms with west Nile virus provided as a recent example. A number of regional international agreements, not previously mentioned, with applicability to GMM are listed in this chapter including the following:

- The ASEAN Agreement on the Conservation of Nature and Natural Resources (Kuala Lumpur, 1985) requires parties to endeavor to regulate and, where necessary, prohibit introduction of alien species (Article 3[3]).
- The Convention of Conservation of Nature in the South Pacific provides that parties shall carefully consider the consequences of deliberate introduction into ecosystems of species not previously occurring therein (Article V [4]).
- The Convention for the Conservation of Biodiversity and the Protection of Wilderness Areas in Central America (Managua, 1992) that requires the adoption of mechanisms to control all exotic species, which threaten ecosystems, habitats, and wild species (Article 24).
- The International Health Regulations (IHR) (Geneva, 1969, as amended, 1982) were adopted by the World Health Assembly of the World Health Organization. They are designed to insure maximum security against the spread of infectious diseases to humans.

An “Overview of Existing International/Regional Mechanisms to Ban or Restrict Trade in Potentially Invasive Alien Species” (Shine, 2006) summarizes: “Globalization provides vastly expanded opportunities for species to be transported to new locations through a wide range of pathways. Those alien species that become established and spread can have serious implications, not just for the environment and communities, but also for national trade and development…Prevention measures should be applied to pathways for introduction and be internationally or regionally coordinated.”

A report by the PEW Charitable Trust entitled “Bugs in the System” (PEW, 2004) made the following statements concerning GM insects: “Genetically modified insects may offer public health and agricultural benefits, but clear regulatory oversight is lacking…It is not clear which legal authority would apply or whether the agency involved would have the tools it needed to assess and manage the risks involved.” This report concludes that the USA federal government lacks a coordinated regulatory approach to ensure that all GM insects are reviewed for potential environmental, agricultural, food safety, and public health risks and that the international regulatory regime for approving such releases is not at all clear.”

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