Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission

World Health Organization,
Geneva, Switzerland, 4-6 May 2009

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Technical consultation on current status and planning for future development of genetically modified mosquitoes for malaria and dengue control

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WHO Library Cataloguing-in-Publication Data:

Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission.

1.Animals, Genetically modified. 2.Mosquito control - methods. 3.Insect vectors - genetics. 4.Dengue - prevention and control. 5.Malaria - prevention and control. I.World Health Organization. II.UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. III.Foundation for the National Institutes of Health (U.S.).

ISBN 978 92 4 159923 8 (NLM classification: QX 600)

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Design and layout by Philippe Casse and Lisa Schwarb
Cover picture: WHO/TDR/Stammers

Printed in Switzerland

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Glossary

APHIS: Animal and Plant Health Inspection Service, Department of Agriculture (USA)
CBD: Convention on Biological Diversity
CI: cytoplasmic incompatibility
COP: Conference Of the Parties
CP: Cartagena Protocol
CSIRO: Commonwealth Scientific and Industrial Research Organisation (Australia)
DDT: dichlorodiphenyltrichloroethane
DEC: disease endemic country
EA: environmental assessments
EIP: extrinsic incubation period
EIS: environmental impact statement
EPA: Environmental Protection Agency (USA)
ERA: ecological risk assessment
ERMA: Environmental Risk Management Authority (New Zealand)
ESC: ethical, social and cultural
FNIH: Foundation for the National Institutes of Health
GM: genetically modified
GMAC: Genetic Modification Advisory Committee (Malaysia)
GMM: genetically modified mosquito
HEG: homing endonuclease gene
IIT: incompatible insect technique
IMR: Institute of Medical Research (Malaysia)
IPCC: Intergovernmental Panel on Climate Change
IPPC: International Plant Protection Convention
IVM: integrated vector management
LM: living modified (mosquito)
NBB: National Biosafety Board (Malaysia)
NEPA: National Environmental Policy Act of 1969 (USA)
NIH: National Institutes of Health
NRE: Ministry of Natural Resources and Environment (Malaysia)
PHS: public health service
RFA: Request for application
RIDL: Release of insects carrying a dominant lethal gene
SIT: sterile insect technique
USDA: US Department of Agriculture
VBD: vector-borne disease
WHOPES: WHO Pesticide Evaluation Scheme

Executive summary

The use of genetically modified mosquitoes (GMMs) for disease control has social, economic and ethical implications, so it is important that the World Health Organization (WHO) and its partners provide guidance to countries on these issues. In collaboration with the Foundation for the National Institutes of Health (FNIH), TDR has developed a series of planning meetings on *Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission*. These technical and public consultations will focus on current status and planning for future development.

The first technical consultation on genetically modified mosquitoes for malaria and dengue control was held at WHO headquarters in Geneva, Switzerland in May 2009. The meeting was attended by 38 scientists and specialists from 13 countries. Its main objectives were to update participants about progress made; to identify issues, challenges and needs; and to make recommendations on how to develop internationally acceptable guidance principles for GMM testing. Discussions focused on the requirements for safety and efficacy testing for human health and the environment, on selection of locations and conditions appropriate for field testing (including regulatory requirements and community engagement) and on needs for communication with end-users and stakeholders. This report summarizes the issues covered and outlines the meeting outcomes. It highlights progress made and recommends how to address the issues, challenges and needs identified during the meeting.

GMM approaches under active investigation for control of malaria and dengue transmission were reviewed. These include: 1) population suppression, defined as reducing numbers of disease-transmitting mosquitoes without affecting the transmission capability of remaining individuals (e.g. through individual sterility); and 2) use of transmission-inhibited populations, in which *Aedes* and *Anopheles* populations have a high proportion that are unable to transmit malaria-causing or dengue-causing pathogens because of population gene replacement. Much progress has been made in recent years, and several of these strategies have achieved proof-of-principle in laboratory studies. A GMM version of the sterile insect technique (SIT) for *Aedes aegypti* is moving to caged field trials, and a GMM version of SIT for *Anopheles gambiae* may progress to caged field trials in coming years. Other GMM strategies, including self-sustaining technologies to achieve long-term transmission control, are anticipated to advance to field testing in the near future.

To update participants on alternative (non-GMM) approaches, speakers involved in developing such technologies were invited to review the progress of two biocontrol methods. Classical radiation-induced SIT for *Anopheles arabiensis* is expected to enter open field trials soon, and *Wolbachia*-mediated biocontrol of *Aedes aegypti* is already undergoing caged field testing. Approaches to testing and evaluation of these alternative non-GMM technologies may help efforts to develop GMM technologies, since they share common aspects with regard to rearing and releasing mosquitoes as well as with regard to monitoring efficacy.

While various GMM development approaches share some issues, they also present different challenges specific to individual products and applications. This consultation addressed practical and technical issues related to the testing of GMM technologies. Although aspects of GMM development and deployment may be governed by established national and international guidelines, regulations and laws regarding recombinant DNA, biological safety, biocontrol and/or pesticides, some features of the envisioned technologies fall outside of existing regulatory schemes. Thus, guidance principles for safety and efficacy testing are needed urgently for when GMM products move from the laboratory to the field.

The main recommendation of the technical consultation meeting was that a working group be charged to produce a guidance framework for the evaluation of GMM for malaria and dengue control. Based on existing literature, regulations and experience, the working group will propose quality standards for assessing safety and efficacy. It will also address ethical, legal, social and cultural issues during the design, conduct, recording and reporting of all phases of GMM field trials prior to deployment. The guidance framework is intended to foster standardization of procedures, comparability of results and credibility of conclusions with regard to independent testing (without conflicts of interest) of various GMM strategies. Compliance with the principles proposed in the GMM guidance framework document should assure that technical and ethical standards have been adhered to within trials, and thus facilitate countries' decision-making regarding GMM as a public health tool for malaria and dengue control.

Included in the main recommendation of the meeting is the development of a communication plan that promotes transparency of the processes used to produce, regulate and use GMM. As part of this plan, an open review activity should be designed and implemented to make the deliberations and decisions of the working group available for comment by scientists, officials, non-governmental organizations, the media and other interested persons and agencies.

A guidance framework working group has been established and it is anticipated that it will complete its activities within the next year and that a public consultation meeting would be organized thereafter.

1 Introduction

Vector-borne diseases are endemic in more than 100 countries and affect approximately 50 % of the world's population. They are emerging and resurging, and result in an unacceptably high burden of disease that reflects inadequate implementation and/or impact from current control measures. Effective prevention strategies could reverse these trends, and vector control aimed at interrupting transmission is a key component of such strategies.

Research is critical in the design, development and testing of new and improved vector control tools and strategies. In addition to conventional control strategies based on chemical, biological and environmental interventions, new methods building on biotechnological advances and the success of agricultural pest control are being developed for use in addressing human health issues. This research has been sustained with support provided by TDR, the John D. and Catherine T. MacArthur Foundation, the Wellcome Trust, the National Institutes of Health (USA), the Burroughs Wellcome Fund, the Foundation for the National Institutes of Health (FNIH) and other funding agencies. GMM research already has yielded the proof-of-principle demonstration that malaria-resistant and dengue-resistant mosquitoes can be produced, and that induced sterility can be generated. However, the way in which laboratory-derived genetically engineered mosquito strains could be deployed effectively, efficiently and safely in the field remains to be determined.

The need to control major diseases such as malaria and dengue is widely accepted, but many challenges remain unmet. These challenges encompass technical issues; funding and regulatory constraints that influence how rapidly new control tools can be developed; anticipated economic, environmental, health and social trade-offs associated with the use of different control methods; and social and cultural issues expected to influence acceptance of these methods. In the case of a new technology such as GMM, careful planning and preparation is required to address these challenges.

2 Process

2.1 Past progress of consultation and discussion

Following early laboratory achievements, a series of workshops in London, Atlanta and Wageningen (in 2001 and 2002[1,2]) discussed benefits and risks of GMM use. Recommendations were proposed regarding laboratory-based research, translational research, safety and efficacy research and communication and education research. The workshop attendees agreed that in order to apply genetic tools to control vector-borne diseases, there is a need to develop consensus regarding key areas that integrate laboratory and field research and address the social, ethical and legal aspects of using genetics-based methods to control vector-borne diseases. A workshop organized in Nairobi (2004[3]) was a specific response to this recommendation, and its report proposed a research agenda to address issues and challenges of genetic control of disease vectors and to develop a strategic plan bridging laboratory and field research.

For two decades, TDR and its partners have advocated for development of GM malaria and dengue vectors to interrupt pathogen transmission. Recently, the Grand Challenges in Global Health (GCGH) Initiative became a major funder of the research effort to develop genetic and biologic control strategies. A working group of scientists later developed recommendations for contained field trials with GMM strategies involving gene drive.[4] In the context of its new vision and strategy, TDR is focusing on requirements for potential field deployment of GM malaria and dengue vectors (e.g. development of guiding principles and capacity-building for site-selection and characterization, assessment of biosafety and efficacy, and ethical, social and cultural considerations). Recent studies on RIDL (release of insects carrying a dominant lethal gene) *Aedes aegypti* mosquitoes in Malaysia stimulated requests from countries for WHO guidance on GMM use [5].

While GM foods have generated considerable debate, public opinion has generally been more supportive of research into genetic modification of mosquitoes to combat deadly diseases.[6] At the same time, developing countries need to systematize the technical, safety and ethical review of trials involving GM insects.

2.2 Current WHO/TDR/FNIH consultation and projects

In the United States of America (USA) and its neighbouring countries, a North American Plant Protection Organization standard, ratified in October 2007, provides guidance regarding confined field trials of GM insects.[7] This adds to the guidance developed by US Department of Agriculture Animal and Plant Health Inspection Service (APHIS) for those seeking permission to field-test GM insects.[8] The International Plant Protection Convention (IPPC) has a related standard on deployment of beneficial organisms; this does not include living modified organisms.[9] While there are currently no WHO guidelines in the global arena regarding GMM trials or regulation, TDR and other WHO departments and partners have joined together to provide guidance supporting countries in addressing safety, ethical, legal and social considerations regarding GMM.

The steps being taken include a series of planning meetings on *Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission*. These technical and public consultations on current status and planning initiate a process for gathering evidence to develop and apply best-practices guidance for countries addressing issues related to genetic modification methods to control vector-borne diseases.

The first technical consultation on *Current status and planning for future development of genetically modified mosquitoes for malaria and dengue control* was held by WHO and FNIH in May 2009 in Geneva, Switzerland (covered in this report). This consultation was intended to extend the process begun in previous meetings by addressing practical and technical issues related to testing of GM technologies within the context of a wider series of consultations that will address other factors affecting their use.

2.3 Meeting objectives and expected outcomes

This consultation was intended to:

- provide an update on current GMM and non-GMM research, including different approaches and plans for testing of efficacy and biosafety for human health and the environment;
- develop plans to address ethical, legal, social and regulatory needs;
- develop strategies for interactions between the developers of the various technologies and potential user communities;
- provide recommendations for internationally-accepted guidance principles for testing GMM technologies.

While various technical GMM development approaches may have issues in common, specific applications present varying challenges. The consultation focused on practical and technical issues associated with moving new GMM technologies from the laboratory to field testing, including:

- technical requirements for efficacy and safety testing for human health and the environment;
- selection of locations and conditions appropriate for field testing, including regulatory requirements;
- requirements for engagement and communications with the communities to be affected by the trials.

The expected outcomes of this first technical consultation were:

- identification of issues and challenges associated with biosafety and efficacy testing, ethical, legal and regulatory issues, and mechanisms for interactions among researchers and the potential user community;
- recommendations for addressing the issues and challenges identified, and for development of guidance principles for testing of GMM;
- publication of meeting proceedings by WHO and FNIH (this report).

3 Current status

Vector control using GMM technologies encompasses multiple approaches. GMM applications seek to suppress or manipulate mosquito populations so as to decrease vector mosquito populations or reduce their ability to transmit disease. These applications release reared mosquitoes into the environment (as do other control strategies not using or applying GM technology to mosquitoes). These technologies are novel in mosquito control, and in the case of population manipulation represent untested concepts for vector interventions in the field. Some non-GMM control approaches are described here alongside GMM where there are common principles of rearing and releasing mosquitoes *(Table 1)* and common measures of efficacy are likely to apply. As this report focuses on GMM technologies, it is not intended that biosafety of non-GMM technologies be approached the same way. However, approaches to testing and evaluation of these alternative technologies may inform the development of GMM technologies. Each approach is proceeding autonomously and will provide information relevant for a guidance framework for conduct of trials. All of these technologies are in laboratory development for mosquitoes; cytoplasmic incompatibility (CI), classical SIT and RIDL are rapidly approaching the open field testing stage.

Table 1. Strategies using GMM and non-GMM technologies

Strategy	GMM technologies	Non-GMM technologies
Population manipulation or replacement	Homing endonuclease	*Wolbachia*-mediated heritable biocontrol
	Medea-based gene drive	
	Underdominance gene drive	
Population suppression (sterile insect technique)	RIDL dominant lethal gene systems	*Wolbachia*-mediated cytoplasmic incompatibility (CI) Classical radiation-induced sterility

4 Issues, challenges and additional needs

There is no widely accepted regulatory or biosafety framework that provides guidance for all aspects of the implementation of GMM technologies. While national standards will necessarily take priority over any other guidance, a framework of guiding principles as recommended here could provide helpful advice to stakeholders. Such a document could foster standardization of procedures, comparability of results and credibility of conclusions, which will be useful for decision-making by funders and end users of the technologies.

Similar guidance exists for genetically modified plants, human vaccines, drug trials, pesticide registration processes and evaluation of classical biocontrol organisms. For example, guidance on clinical drug trials has led to the emergence of a harmonized internationally recognized process. The WHO Pesticide Evaluation Scheme (WHOPES)[10] system may serve as an additional template for GMM guidance.

Technical needs include:

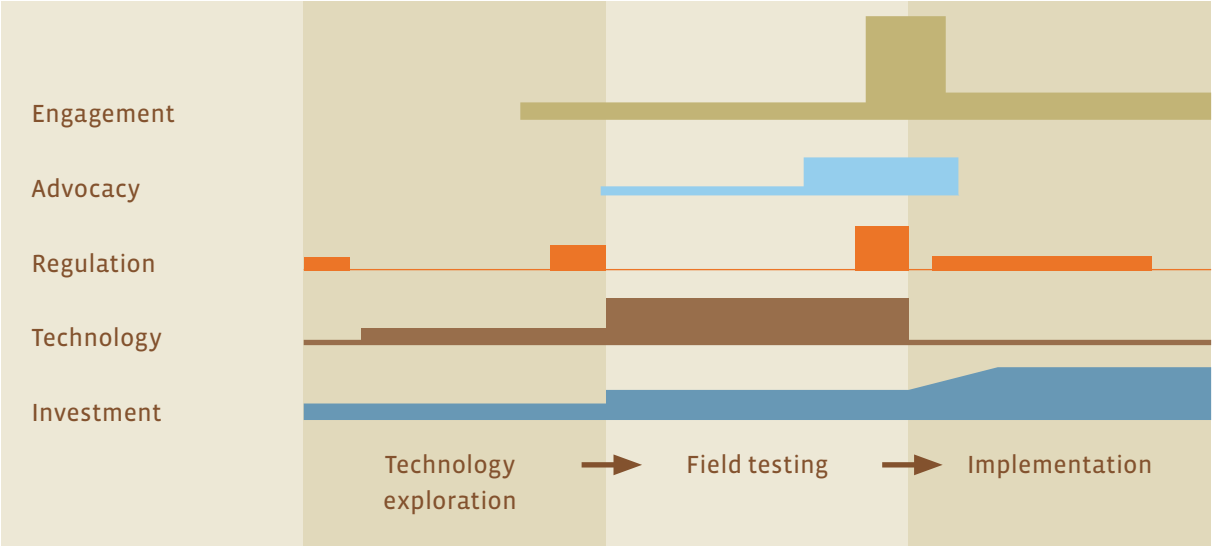
- **Risk analysis** – Identify methods to define hazards and prioritize risks to be monitored, starting at the laboratory phase and continuing through field release. These include additional approaches to identifying environmental and ecological hazards such as fault-tree and event-tree analyses.[11] Community engagement to identify valued aspects of the environment should be used to inform this process. Integrated methods for risk analysis should be developed to ensure that novel and conventional control approaches are judged by common standards.
- **Models to extrapolate limited testing to wider field applications** – Create models that relate measurable entomological outcomes to epidemiologically-relevant impacts.
- **Performance measures** – Define useful performance measurements at different phases, such as: Does the organism persist in the field? Is the critical phenotype stable? Is there an epidemiologically relevant impact? How can endpoints, such as transmission-blocking and vector or pathogen elimination, be measured in the field?
- **Production and release systems** – Develop methods for efficient production and release of mosquitoes.
- **Quality control standards** – Develop standards for quality control, including genetic characteristics during scale-up and delivery, and develop procedures to measure quality on a time scale that allows management response for large-scale production and release.

Ethical, social and cultural (ESC) needs include:

- **Standardized approaches** – Experts support standardizing regulation and good practices to best fit the real problems. The European Union and the Convention on Biological Diversity (CBD)/Cartagena Protocol (CP) are developing such guidance. Although these processes may go faster than the proposed guidance framework working group and may set precedents, working with these groups could establish a harmonized approach.
- **Articulation of disease endemic countries (DECs)-focused needs** – The full range of issues and problems faced by DECs are unclear, as are the extents to which local and regional differences will require regulatory differences. One guide may not "fit all" cases in which different approaches are needed should be defined clearly.

- **Integration of end-user concerns throughout the biosafety process** – Biosafety regulation cannot be assumed to be "fair" or "just" without the core consideration of human protection being included. "Safety" implies a value judgment because it suggests there is a technical level of risk that is acceptable. One way to ensure ownership – and useful input from end-users – is to form partnerships during research and development of technologies and biosafety information.
- **Catalysts for national regulation** – What will spur the development of regulations within DECs, and who will be responsible for this? Each country will address its own requirements, but all can draw on internationally-accepted guidance. Developing this guidance is WHO's role after the framework is prepared.
- **Ownership** – The development of GMM technologies will have greater local ownership if conducted through mutual participation. Broad inclusion and transparent processes support ownership and uptake.
- **Values and risk** – Risk reflects real conditions of nature, and social values are used to interpret risk. Values are intrinsic to individuals and communities. Additional information on real biological or ecological conditions or new circumstances may change the parameters of a risk assessment (its likelihood, consequences or confidence level), but it may not change the attitude to the risk.
- **Trade-offs between early and informed response** – It is important to make decisions about perceived risks and opportunities early in the development of a technology. While early decisions can reduce later risks, these decisions will be based on lower levels of information and confidence than those made later. This trade-off should be recognized explicitly and the decision-making process should be flexible enough to allow revision of decisions and procedures as more information becomes available.
- **Understanding the process of engagement** – Engagement evolves *(Fig. 1)*, but can we indicate how? Engagement and advocacy are different actions, but each interfaces with technical development, investment and regulation. Engagement could evolve into advocacy.
- **Planning for success** – Reducing disease in DECs will open opportunities for social and economic change that could have profound effects, not all of which may be positive to all individuals. How can we plan for these changes and gain the greatest advantages for society?

Fig. 1. Development of genetically modified vectors over time



Three phases of development are envisioned: technology exploration, field testing and full implementation as a routine control measure. The height of the bars indicates relative levels of activity, but no absolute reference to different categories is intended. Some activities, such as regulation may be ongoing, but require periodic intense activity to establish standards, legal requirements etc. Technology, on the other hand, plays a prominent role in the first two stages but becomes relatively less significant once routine implementation is accomplished, although improvements in monitoring would continue to be needed. The level of investment required for implementation likely will be dependent upon the particular strategy under consideration. All activities must be coordinated so proper regulation, community engagement and investment are in place as the technology proceeds.

5 Recommendations

Action: Establish a working group to develop a guidance framework document for quality standards to assess safety and efficacy and address regulatory, legal, ethical, social and cultural (ESC) issues during GMM development and testing.

Purpose: To ensure that considerations of safety, efficacy, regulation and ESC issues are consistently and responsibly addressed before and during GMM deployment.

Scope: Disease focus (mainly malaria and dengue, potentially chikungunya and yellow fever); vector focus (*Aedes, Anopheles*); technology focus (population suppression, modification, manipulation or replacement); country focus where testing of GMM may be more imminent.

Content: The framework:

1. Describes phased testing (Appendix 2 shows an example of a phased testing pathway) of GMM that includes stepwise testing for efficacy and biosafety in relation to human health and the environment.
2. Prescribes in general terms:
   - the different levels of safety testing that must be addressed at different phases of testing;
   - how efficacy will be measured for the various strategies under consideration at different trial phases; and
   - requirements for ongoing safety and efficacy evaluation after testing is completed.
3. Reflects the fact that measures of efficacy will vary depending upon the strategy and disease system.
4. Considers anticipated ecological contexts (e.g. rural, urban).
5. Provides operational definitions for critical terms such as "refractory" and "community".
6. Considers planning requirements for remediation/recall.
7. Recommends appropriate containment/confinement measures to be in place during early phases.
8. Describes monitoring plans for desirable and undesirable outcomes.
9. Recommends oversight by an independent safety review group designated by the host country for each phase of testing. This will vary by country but should resemble the Institutional Biosafety Committee that NIH guidelines specify[12].
10. Recommends investigators devise and implement standard operating procedures for evaluating GM mosquito product efficacy before moving to large field cage trials.
11. Recommends that each GMM product must proceed through a risk assessment process, which will necessarily require a case-by-case approach.
12. Categorizes risks that may result from various GMM products/applications; such an assessment would be of use to scientists and regulators.
13. Develops models for effective stakeholder/community engagement from an early stage.
14. Develops guidance for human subject aspects of confined and open field releases.
15. Assesses local capacity for ethical review and oversight and develops a plan for current needs.
16. Takes into account a review of existing regulatory approaches.

Outcome: A guidance framework document to foster standardization of procedures, comparability of results and credibility of conclusions in addressing the technical requirements for decision-making by countries about the use of the GMM control methods as public health tool for dengue and malaria control.

6 Next steps

WHO-TDR is to liaise with uptake targets (WHO regional offices and DECs) to ask the following:

- What they would do with the outputs of this process?
- What are their interests?
- What are the necessary preparatory steps and capacities (legal, technical, infrastructure, ESC, site selection) for uptake?

A working group should be established in response to this. WHO should attempt to select working group members who have knowledge of the subject but are free from conflicts of interest to encompass a broad array of expertise in their charged areas, for example in efficacy (epidemiology, biological modelling, medical entomology, virology, parasitology, product development); safety (ecology, genetics, environmental and human/animal health risk assessment, invasion biology, population biology, evolutionary biology); regulatory (nation-specific, international, legal, public good principles); ESC (anthropology, sociology, bioethics, legal, institutional, economics); and communication (general, scientific, media).

Working group commissions should integrate technical/ESC documents from suitable experts, who may or may not be working on GMM strategies, to:

- review reports from previous meetings on GM vector technology (London/Atlanta, Wageningen, Nairobi);
- conduct case studies of countries most likely to have considered regulatory issues surrounding GM insects and see how they have handled applications: for example, whether countries have adapted or customized the use of Cartagena Protocol (CP) Annex III (risk assessment) for their own national decisions about GM insects, and how regional cooperation affects national approaches on GM insects;
- review existing related work on biocontrol and biopesticide regulatory frameworks, international agreements on release of organisms and recent reviews for relevant information, lessons and directions;
- form a formal or informal link with the Convention on Biological Diversity (CBD) Conference Of the Parties (COP)/Cartagena Protocol (CP) group looking at GMM risk assessment to nominate experts or establish a collaboration mechanism;
- convene working group meeting(s) and prepare framework document.

The working group should provide a comprehensive two-way communication plan that ensures transparency, including:

- English and other languages used appropriately, with technical language suitable for both scientific and lay audiences;
- accessible publication and dissemination of the framework;
- public consultation that uses working group outputs (for instance, considering different plans or scenarios);
- communication to scientific community (including in DECs), NGOs, media; and
- iteration mechanism(s) to respond to feedback following public consultation or other public input.

Finally, the framework document will be submitted to WHO and partners and to interested DECs. This framework will be reviewed and updated as circumstances change.

7 References

1 Alphey L et al. Malaria control with genetically manipulated insect vectors. *Science*, 2002, 298:119–121.

2 *Proceedings of the Frontis Workshop on Ecological Challenges Concerning the Use of Genetically Modified Mosquitoes for Disease Control*. Wageningen, Netherlands, 26-29 June 2002 (**http://library.wur.nl/frontis/malaria/index.html**, accessed 16 November 2009).

3 *Proceedings of the Joint WHO/TDR, NIAID, IAEA and Frontis Workshop on Bridging Laboratory and Field Research for Genetic Control of Disease Vectors*. Nairobi, Kenya, 14-16 July 2004 (**http://library.wur.nl/frontis/disease_vectors/index.html**, accessed 16 November 2009).

4 Benedict M et al. Guidance for contained field trials of vector mosquitoes engineered to contain a gene drive system: recommendations of a scientific working group. *Vector-borne and Zoonotic Diseases*, 2008, 8:127–166.

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8 *Transgenic insect permit guidance*. United States Department of Agriculture (USDA) (**http://www.aphis.usda.gov/biotechnology/arthropods.shtml**, accessed 16 November 2009).

9 *Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms*. International Plant Protection Convention (IPPC), 2005 (**https://www.ippc.int/servlet/BinaryDownloaderServlet/76047_ISPM_3_E.pdf?filename=1146657660135_ISPM3.pdf&refID=76047**, accessed 16 November 2009).

10 *WHO Pesticide Evaluation Scheme*. World Health Organization, 2009. (**http://www.who.int/whopes/en/**, accessed 16 November 2009).

11 Ayyub BA. *Risk analysis in engineering and economics*. Boca Raton, USA, Chapman and Hall/CRC, 2002.

12 *Notice pertinent to the September 2009 revisions of the NIH guidelines for research involving recombinant DNA molecules (NIH guidelines)* (**http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc7261582**, accessed 16 November 2009).

8 Working papers presented at the Technical Consultation

Participants at the meeting presented working papers in plenary sessions on the current status of research on genetically modified mosquitoes covering a range of different approaches. These include proposed plans for stepwise testing for efficacy and biosafety, as well as addressing ethical, social, legal and regulatory issues, in the context of "case studies" of specific approaches. The detailed papers presented at the meeting are reported below and references listed together in section 8.16.

8.1 Population suppression strategies for dengue virus vector control *(Aedes)*

Luke Alphey
University of Oxford, Oxford, United Kingdom

Introduction

Population suppression strategies aim to reduce the number of vector mosquitoes in the target area. This is in contrast to population replacement strategies, which aim to alter (reduce) the vector competence of the mosquito population with only incidental effects on the total number of mosquitoes.

Current status

Relatively few genetics-based population suppression strategies have been proposed. Most represent variants on the sterile insect technique (SIT). Several such strategies, including at least one specifically based on use of GM mosquitoes, are either in field testing or expected to move to this phase in the near future.

SIT is a species-specific and environmentally non-polluting method of insect control that relies on the release of large numbers of sterile insects.[1, 2] Mating of released sterile males with native females leads to a decrease in the females' reproductive potential and ultimately, if males are released in sufficient numbers over a sufficient period of time, to the local elimination or suppression of the pest population.
Highly successful, area-wide SIT programmes have eliminated or suppressed a range of major veterinary and agricultural pests around the world. These programmes can succeed on very large scales – the largest rearing facility alone produces around 2 billion sterile male Mediterranean fruit flies per week (~20 tons/week) primarily for use in California and Guatemala. For these pests, SIT is a proven cost-effective strategy for eradication or suppression of target populations, or to protect areas against invasion or re-invasion.

For mosquitoes, the situation is less clear. Field trials in the 1970s and 1980s demonstrated that SIT could also be made to work against mosquitoes, even with the technology then available.[3, 4] For example, *Anopheles albimanus* was successfully controlled in a trial in El Salvador using chemosterilized mosquitoes.[5]

The fundamental properties of SIT are still highly attractive for mosquito control. This has led to a resurgence of interest in recent years, with several research groups trying to mitigate or bypass some of the technical limitations which prevented conventional SIT from becoming a widespread approach following early trials. Two issues in particular may be addressed by genetics.

Sterilization

Recent advances allow several potential improvements over the methods available in early trials. All current SIT programmes use radiation to sterilize the insects. However, it has proven difficult to irradiate mosquitoes to near-complete sterility without significantly weakening them.[6, 7] Two groups are trying to develop radiation-based SIT for mosquitoes with some success currently led by Romeo Bellini (Italy, *Aedes albopictus*) and Mark Benedict (IAEA in Austria, primarily *Anopheles arabiensis*).[8, 9]

Early mosquito SIT trials used a range of sterilization methods, for example chemosterilants in the case of the *Anopheles albimanus* programme in El Salvador. Cytoplasmic incompatibility (CI) was also used successfully in field experiments with *Culex pipiens fatigans*.[10] CI is caused by maternally transmitted intracellular bacteria of the genus *Wolbachia* and refers to the strongly reduced hatch rate in matings between infected males and females that are either uninfected or harbour a different infection type.[11] Therefore, infected males released into an uninfected population are sterile without need for radiation. Interest in this incompatible insect technique (IIT) has recently revived.[11,12] However, infected females are fertile with infected

males, so inadvertent release of infected females could lead to replacement of the target population with a *Wolbachia*-infected form rather than suppression. For a species such as *Aedes aegypti* not naturally infected by *Wolbachia*, this infection could spread beyond the release area, potentially worldwide, and may be irreversible. For a naturally infected species such as *Aedes albopictus*, such replacement should not spread far beyond the release area and is theoretically reversible, if desired, by mass release of wild-type females. Modified strains showing CI with wild type have been constructed for *Aedes aegypti, Ae. albopictus* and *Ae. Polynesiensis*.[12, 13, 14]

Recombinant DNA methods are also being developed for sterilization. It has been proposed that "genetic sterilization" could be achieved by use of engineered insects in a strategy termed RIDL (release of insects carrying a dominant lethal gene or genetic system).[15, 16] Released males would mate with wild females and all progeny would inherit the lethal gene and consequently die. The lethal gene needs to be conditional, so that the strain can be bred under permissive conditions before release. Adjusting the time of death from the embryonic lethality characteristic of other methods to later lethality, perhaps pupal, could be advantageous to mitigate the effects of density-dependent population dynamics thought to be characteristic of some mosquito species such as *Aedes aegypti*.[15, 17] It has also been suggested that by restricting the lethal effect to females, both sex separation and genetic sterilization could be provided by the same genetic system.[15, 16]

Sex separation

It is highly preferable to release only sterile male mosquitoes, not a mixture of males and females. Sterile females may bite and potentially transmit disease, and may also distract co-released sterile males from seeking out wild females.[18] For *Culicines*, sex-separation has been achieved by physical methods based on pupal size, with 99–99.9 % accuracy.[19, 20] Several strains suitable for genetics-based separation have been constructed using classical genetics. These were based on the translocation to the Y chromosome of a selectable marker; for example, insecticide resistance.[21]

It has been proposed that engineered repressible female-specific lethal genes could provide effective genetic sex separation.[15, 16] Such strains have been developed for Mediterranean fruit fly[22] and for *Aedes aegypti* (Alphey, unpublished). Another proposed use of recombinant DNA methods is to engineer sex-specific expression of a visible marker, such as a fluorescent protein, allowing fluorescence-based sorting.[23, 24] This method may be limited by the speed of available fluorescence-based sorters.

Other traits and approaches

Several other useful traits are available through genetic engineering, such as a heritable marker to distinguish engineered and wild-type insects, and various ways of mitigating accidental releases of insects.

Driving lethal genes into populations

On the border between population suppression and population replacement is the notion of driving deleterious genes into the target population. Methods to do this have been proposed using homing endonuclease genes (HEGs).[25, 26, 27] These would tend to spread and impose a genetic load on populations; mathematical models suggest that this might lead to extinction. The same approach could be used to drive anti-pathogen effectors or mutations; the issues and challenges around

this approach are much more akin to population replacement. It also has been suggested that HEGs could be used to provide "RIDL with drive": an augmented version with greater sterility gene persistence in wild populations, though without the long-term persistence or spread characteristic of most population replacement approaches.[27]

Issues

Some of these issues are common to many control strategies, including other genetic control strategies.

- Intervention is species-specific:
  - Multiple vector species in target area?
  - Mating barriers between populations and/or cryptic species?
- Relationship between number of mosquitoes and epidemiology may not be clear:
  - Population suppression aims to reduce the number of vectors, but what level of success is required to produce a given level of benefit (reduction in human morbidity or mortality)?
- Community engagement:
  - There is likely to be some level of disquiet about any release of mosquitoes, as sterile-release methods require release of large numbers of insects.
  - Use of modified mosquitoes may induce additional concerns.
  - The properties (phenotype) of the modification (self-spreading or self-limiting) should be the primary consideration for risk assessment. However, the means by which the strain was constructed – by radiation, artificial infection with *Wolbachia* or recombinant DNA methods (genetic engineering) – may also influence public perception and in many countries will affect or define the regulatory pathway.
  - Communicating mosquito and disease biology, and the risks and benefits associated with specific novel control strategies, is resource-intensive and has no obvious endpoint.

Challenges

Economic cost-benefit analysis, which is needed to support use of novel interventions, is difficult because of lack of reliable data on the economic burden of disease for dengue and other neglected tropical diseases, and because of uncertainty around development and implementation costs. Ideally it would be possible to analyse not only the cost-effectiveness of the stand-alone novel strategy, but also to compare it with existing alternatives and to model its incorporation in integrated vector management (IVM) programmes, and indeed integrated disease management programmes including drugs and vaccines, where available.

As genetics-based population suppression moves from laboratory to field, the lack of a clear regulatory framework for field use of modified mosquitoes is a significant challenge. This issue is not restricted to developing countries, or to strategies dependent on the use of recombinant DNA technology. Once regulatory frameworks are in place, risk assessments and public consultation also will be lengthy processes due the novelty of technologies and lack of experience by regulating agencies. The route to implementation of control programmes based on these technologies is not obvious. Agricultural SIT programmes have generally been established and operated by governments, though there is limited private-sector involvement. Existing vector control programmes are generally government-funded and -operated, though they purchase vector control products and services from the private sector. The development of new vector control approaches is generally in the private sector. The current genetic-based technologies are perceived as too high-risk for large companies to bring them into their portfolios. This risk is a combination of the technical and regulatory risks of bringing the technologies to market and the market risk or uncertainty regarding customers and prices. For example, the NGO sector has encouraged the private sector to develop and produce bednets to prevent malaria by guaranteeing

certain purchase quantities. This model does not exist for novel strategies and dengue. There is one small private-sector entity involved today but the technologies need to be "de-risked" before larger companies get on board and facilitate large-scale implementation. This de-risking could be in the form of funding for development and/or in conditional purchase guarantees.

Additional needs

In addition to the issues and challenges mentioned above, several other areas should be addressed.

Field demonstration

While there is much work to be done in the laboratory, the pressing need is for field data on the performance, effectiveness and cost-effectiveness of various population suppression strategies for which prototype strains have been developed. Convincing demonstration of suppression of wild target mosquito population(s) by one or more of these strategies would be transformative.

Improved large-scale rearing methods and cost estimates for these

Implementation costs of genetics-based population suppression strategies depend in large part on the costs of rearing and distributing sterile mosquitoes. While methods exist from early trials, there is considerable scope for improvement and cost reduction.

Better understanding of mosquito (especially male mosquito) ecology and behaviour, and the effect of vector numbers on epidemiology of relevant diseases

Guidance will be developed for the use of modified mosquitoes in genetics-based population suppression strategies. This guidance should cover the full spectrum of proposed modified mosquitoes.

The self-limiting nature of sterile insects (whether sterilized by radiation, *Wolbachia*/CI (IIT), or RIDL genetic engineering) tends to make the issues related to field use of these somewhat less challenging than for self-spreading systems characteristic of population replacement strategies (including *Wolbachia*- and gene driver-based replacement strategies). They also are closer to field use, so might be appropriate to consider first. WHO/TDR funding for capacity-building and guidance development and this technical consultation are all steps in the right direction.

Recommendations

Recommendations relate to meeting the issues, challenges and additional needs identified above. More generally, we are on the brink of having a suite of new tools for field testing and, ultimately, programme use. The development costs and timescales are attractive relative to other approaches such as new drugs, vaccines or insecticides, yet they are beyond the reach of individual research groups. Though there is currently considerable momentum behind the development of genetic control methods, this requires ongoing support, particularly during the transition from laboratory to field and into the first large-scale implementation programmes.

8.2 Conventional sterile insect technique against *Anopheles arabiensis*

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Project description

This project uses conventional sterile insect technique (SIT) against the mosquito *Anopheles arabiensis*. The project is composed of two parts: technology development and field feasibility studies. The International Atomic Energy Agency (IAEA) is primarily involved in technology development, but it also plays a supporting role assisting national efforts in their field feasibility studies.

Two field sites are being studied for the initial feasibility studies: the Nile valley in the Northern State of Sudan and Reunion Island. In both instances, *An. arabiensis* is the sole malaria vector.

Current status

The technological aspects include four components: sex separation methods, sexual sterilization using gamma irradiation, mass production and release methods. We expect to have at least rudimentary methods for performing all of these by the end of 2009. Sex-separation is based on a translocation which links dieldrin resistance *(Rdl)* to the Y chromosome. The relationship of irradiation to sexual sterilization has been described, and to a large extent studies of its effect on male competitiveness have been completed. Mass production trays, cages, racks, pupa separation and blood-feeding apparatus are undergoing construction, modification and refinement.

The Sudan SIT project is designing a production facility and trial releases are under way. Extensive studies of the distribution of larval breeding sites have been conducted; these will be used to determine the seasonal dynamics of *An. arabiensis* that will be included in a model of adult populations.

The project has been reviewed by the Sudan National Biosafety Framework, which has expressed no prohibitive safety concerns about the activities.

The Reunion Island project has just entered into studies of wild mosquito abundance and genetics as a preface to determining whether to proceed with a feasibility study. No specific plans for a production facility or release numbers is expected for at least three years.

Issues

In the context of this meeting, there are two issues that have not been satisfactorily addressed, but neither is unique to this technology: (1) if the programme eliminates *An. arabiensis*, what will be the long-term consequences of creating a malaria-naive population? (2) Will the introduction of an *Rdl* insecticide resistance allele make conventional control more difficult? This is being addressed by laboratory experiments to some extent.

Challenges

A myriad of logistic, financial and technical challenges face these programmes though not particularly related to human health or regulation. To test any technology on a large scale with little experience in a developed country is difficult; to do so in a developing country is more so. The availability of trained personnel, materials and infrastructure present country-specific difficulties. Some of this cannot be anticipated and may not be easy to remedy.

Technical challenges of production

The technical challenges of SIT have been met and solved to a limited degree by previous SIT programmes. These used techniques that increased the scale of laboratory culture, but the specialized devices they developed are no longer available. Therefore, the IAEA programme is attempting to learn from previous efforts to develop improved versions using modern knowledge and materials,

and to develop technology that is scalable to larger production numbers. Thought is being given to mechanization, cost, efficiency and, secondarily, use with *Aedes* spp. The equipment has not been completed and tested, so there is no certainty that the current systems will be suitable.

Field feasibility studies challenges

The density of mosquitoes in Sudan is sufficiently low that it is difficult to obtain good estimates of adult populations. Therefore, two years of monthly larval surveys conducted in 2005–7 will be the basis for estimating adult populations. This is a significant but not insurmountable biological modelling problem.

Aerial release technology is not available for mosquitoes, but for the first releases in Sudan we believe that ground and river bank releases of pupae will be suitable. Devices for doing this must be created and tested. On Reunion Island, it seems certain that aerial release will be necessary since the terrain is uneven, complex and somewhat inaccessible by ground vehicles. Aerial release technology developed for other delicate insects must be adapted for mosquitoes.

Additional needs

Planning vector release programmes will be facilitated by development of general mathematical models of SIT that are updated and modified to include characteristics such as larval density dependence and survival, reduced mating competitiveness, species bionomics and semi-sterility. These programmes should be accessible via a simple interface to end-users who are planning release programmes, not only software developers or modellers.

Surveillance methods for *Anopheles* at very low population densities will be challenging. During the eradication of *An. arabiensis* from Egypt, the absence of rebound of populations to cessation of control efforts confirmed elimination, but this will not be suitable for routine assessment of programme effectiveness. Sensitive methods and materials such as attractants are needed.

Expert independent ethicists and public health practitioners should resolve several ethical and environmental issues that have been raised. These include the desirability of inevitably creating naive human populations, the desirability of area-wide control methods in an environment in which community-based interventions are popular, eradication of genetic forms and defining situations when informed consent is and is not applicable.

Recommendations

1. Provide funding to develop the general mathematical model described above. While there are several efforts to develop models for specific technologies, these are neither sufficiently flexible nor accessible by project planners to make them of value for routine programme implementation.
2. Provide funding for sensitive methods for *Anopheles* surveillance. These must be robust and capable of being applied over large areas.
3. Provide funding for development of aerial release equipment. The same equipment can likely be used for all genetic release programmes. Such equipment allows the spatial extent of vector releases to be realistically considered.
4. Create a panel of experts to develop a landmark document of conclusions regarding ethical and environmental issues such as those described above. This panel must be of high scientific calibre, completely independent and recognized by international bodies such as WHO.

8.3 Using inherited *Wolbachia* endosymbionts as a biological control approach to eliminate dengue transmission

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By introducing into *Aedes* mosquitoes known strains of inherited *Wolbachia* endosymbionts we can selectively kill old adults in the mosquito population. With these individuals removed, the population's ability to transmit disease to humans can be greatly reduced or even eliminated due to the length of the extrinsic incubation period (EIP) of dengue virus within a mosquito. Moreover, the inherited *Wolbachia* endosymbionts that can shorten lifespan can actively invade populations they infect through cytoplasmic incompatibility. As such, they provide their own genetic drive system. The strengths of this approach are discussed below.

This approach is being developed within a biological control framework and will not require the release of transgenic insects into the environment, raising its acceptability among communities and regulatory authorities. In addition, *Wolbachia* endosymbionts are very common in natural insect populations, including many species of pest mosquitoes that commonly bite people but are not major disease vectors.

This approach, like most biocontrol approaches, can be considered very "green" with its very low impact on non-target organisms, including humans, compared to current insecticide-based control programmes. This improves its acceptability, which is borne out in our current social research that shows community attitudes to the approach are very favourable.

This approach is compatible with current methods of dengue control and can be implemented in a seamless way with existing approaches. Moreover, the programme should be very inexpensive over the medium term compared to existing approaches. The *Wolbachia* approach will allow the paradigm of dengue control to move from outbreak management to prevention. It will be particularly useful in city areas, where control is very difficult and community participation approaches are least effective.

State of technology

- Life-shortening *Wolbachia* have been successfully introduced into *Aedes aegypti* laboratory lines where they are inherited, invade laboratory colonies and generate a 50% reduction in adult lifespan.
- Before *Wolbachia*-infected female mosquitoes die, they show altered feeding behaviour and are unable to successfully bite, enhancing the strategy.
- *Wolbachia*-infected mosquitoes are unable to support a dengue virus infection, which provides an alternative control point in addition to life-shortening.
- *Wolbachia*-infected *Aedes* eggs do not survive dry diapause as well as wild eggs, which can act to reduce population sizes of mosquitoes in areas where dry diapause is ecologically significant.
- Effects seen in inbred laboratory lines are also seen in outbred Australian *Ae. aegypti* genetic backgrounds.
- Data from laboratory characterization experiments when used to parameterize models suggest *Wolbachia* will be able to invade field populations if an initial release ratio of approx. 0.4 can be exceeded. *Wolbachia* will not spread in an uncontrolled manner, however, due to the high threshold that needs to be exceeded and the low dispersal ability of *Ae. aegypti*. This model is currently being validated in large contained field enclosure experiments.

- Through anthropological research, we have determined broad community acceptability of the strategy and also issues of concern to communities. We have begun to address these issues of concern; for example, experiments are conducted to demonstrate that *Wolbachia* is not present in mosquito saliva and that laboratory volunteers that routinely bloodfeed *Wolbachia*-infected colonies do not have antibodies against *Wolbachia*.

Proof of principle

We consider that proof of principle will be met once a successful open release in a geographically restricted area has demonstrated that *Wolbachia* can invade the local mosquito population and produce the desired life-shortening and anti-viral traits in the target population. Due to the sporadic nature of dengue outbreaks in time and space, we do not think it realistic for this release to obtain a direct measure on disease transmission alteration. However, it will provide the required data for epidemiological models that will predict the likely outcome on disease transmission. A direct test on disease transmission will need to be undertaken in a subsequent larger-scale release in an endemic area of high transmission intensity, such as central Viet Nam. Our intention is for the first proof of principle release to be undertaken in "our own backyard" in Australia, where the regulatory environment is relatively sophisticated and there is a strong history of biological control interventions. In Australia's recent history, over 100 pest species have been targeted with government-regulated open releases of foreign biological agents. Some of these programmes have been iconic successes, such as the *Cactoblastis* moth targeting introduced *Opuntia* prickly pear, and in more recent years the release of multiple viral pathogens to successfully control European rabbit populations. Next we envisage a pilot open release staged after the first Australian release in an ecologically different context in Viet Nam and/or Thailand to complement the release in Australia.

Challenges

While Australia has a relatively sophisticated regulatory framework, our project does not fit neatly into that framework. As such, it could be interpreted under a tight reading of Australian law that a pilot release does not need regulatory approval, which is not what we would like. To overcome this issue, we have engaged scientists at Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) to develop a risk assessment of the proposed intervention and then to have this endorsed by a panel of independent experts. This risk assessment will then be used in meetings with Australian government regulatory officials to guide government assessment of the pilot releases.

While we can get indications of how *Wolbachia* will behave in an open release from cage experiments, we cannot entirely predict it. At some point we have to have sufficient confidence to push ahead with an open pilot release and learn from that experience. We feel that we should proceed to this step if we can demonstrate that the *Wolbachia* infection will invade a large cage population. Our new data on dengue blockage makes the life-shortening effects under field conditions of secondary importance.

While community support seems positive overall, how do we decide that it is sufficiently positive to allow an open release to proceed in a given community? Historically in Australia biological control interventions proceed with little regard for community acceptability. However, in this case we are releasing insects that will directly interact with the human population.

8.4 Using bacteria to contain the spread of malaria

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The concept

The unbearable burden of malaria is increasing worldwide and novel approaches to fight this deadly disease are urgently needed. Unlike AIDS and tuberculosis, malaria requires an intermediate vector (the mosquito) for transmission to occur. Recent technical advances in vector biology made possible a new strategy to combat malaria: genetically modifying the mosquito to reduce its vectorial competence. However, one crucial unresolved aspect of this approach is how to introduce effector transgenes, whose products interfere with parasite development in the mosquito, into wild mosquito populations in the field. Several strategies have been proposed, such as *Wolbachia*, homing nucleases and transposable elements. Yet the feasibility of their implementation is a concern. The recent publication of the MEDEA system offers hope but implementation of this strategy is likely to take a long time, perhaps decades.

A significant limitation for the application of all genetic drive mechanisms, including MEDEA, is that vector anopheline mosquitoes commonly exist as reproductively isolated populations (cryptic species). This complicates logistics for gene introgression into wild mosquito populations. Another consideration is that mass mosquito releases must be of males only. While physical and genetic methods for the mass production of male mosquitoes exist, this step adds an extra burden to the approach.

We are exploring an alternative approach to introduce effector genes into mosquitoes. It is based on the fact that as for all higher organisms, the mosquito carries a microbiota in its midgut lumen. Rather than genetically modifying mosquitoes, the proposal is to genetically modify the bacteria that inhabit the mosquito midgut. Several considerations argue in favour of using bacteria to manipulate vector competence.

- Bacteria live in the midgut, the same mosquito compartment where the most vulnerable stages of *Plasmodium* development occur.
- The number of mosquito midgut bacteria increases dramatically with a blood meal (when parasites are ingested), correspondingly increasing the output of the effector molecules that they are engineered to produce.
- Genetic manipulation of bacteria is much simpler and faster than genetic manipulation of mosquitoes.
- Given that the use of multiple effector proteins is essential to avoid resistance, it is straightforward to formulate an efficient multi-effector combination by simply feeding mosquitoes a mixture of GM bacteria expressing different effector genes.
- Bacteria are much easier to introduce into mosquito populations than transgenes. Importantly, this approach bypasses genetic barriers of reproductively isolated mosquito populations (cryptic species) that commonly occur in areas of high malaria transmission and will hinder the spread of mosquito transgenes.
- Bacteria can be produced easily and cheaply in large quantities in disease endemic countries.
- Unlike mosquito transgenes, inactivation of bacterial transgenes after many generations in the field is not a major concern because of the easier logistics of introducing freshly transformed bacteria. Moreover, if an effector gene fails to perform as promised, introduction of alternate transgenes is relatively simple.
- Regulations already exist regarding evaluation of bacteria to be released into the environment.

A major outstanding issue is how to introduce the engineered bacteria into mosquito populations in the field; devising such strategies is our main goal.

Initial steps

A major strength of the GM bacteria (paratransgenesis) approach is that proof-of-principle experiments have already demonstrated the feasibility of using GM bacteria to suppress the vectorial

competence of mosquitoes.[28] Briefly, we have shown that:

- bacteria engineered to express molecules that interfere with *Plasmodium* development (i.e. effector molecules) significantly decrease vectorial competence of mosquitoes; and
- bacteria adapted to live in the mosquito midgut successfully compete with the mosquito's endogenous microbiota.

The recent finding that *Asaia sp.* bacteria may be vertically transmitted[29] greatly increases the promise of this approach because it suggests means for introducing engineered bacteria into mosquito populations in the field.

The next set of experiments will evaluate the different parameters in preparation for future field trials. Such trials will follow all initial feasibility experiments and occur after regulatory, ethical and social issues have been addressed. The subjects will include:

- Effector molecules. The identification of multiple proteins or peptides that arrest parasite development or prevent its invasion of the mosquito midgut is a critical component of this approach. This is important for two reasons. First, a single effector molecule is unlikely to completely block parasite development, while this may be achieved by a combination of effectors. Second, because *Plasmodium* can evolve resistance to the barrier imposed by the effectors, several blocking agents are needed, each acting by an independent mechanism.
- Engineer bacteria to secrete effector proteins. If effector proteins are kept inside the bacteria or attached to their surface, they will be unable to diffuse to their targets (parasites or lumenal surface of the mosquito midgut). To overcome this limitation, bacteria need to be engineered to secrete effector proteins.
- Adapt bacteria for survival in the mosquito. The efficacy of the approach is critically dependent on strong bacteria fitness within the mosquito midgut. It has already been shown that it is possible to adapt bacteria for survival in the adult gut[28] and survival from larvae to adults.[29]
- Microbiota of field mosquitoes in Africa. An important consideration for future implementation of this approach is to determine whether the bacteria species to be engineered naturally occurs in field-caught mosquitoes in Africa. From the biosafety point of view, it will be much easier to justify work with bacteria that naturally occur in the target area. Note that non-recombinant *P. agglomerans* has already been approved by United States regulatory agencies (Environmental Protection Agency [EPA] and US Department of Agriculture [USDA]) for spraying in fruit orchards to prevent fire blight disease.

Further steps

Success for the initial steps above can be measured by 1) the degree that mosquito vectorial competence can be reduced by the engineered bacteria and 2) by the efficiency with which the engineered bacteria compete with endogenous mosquito microbiota. The next phase of this strategy will be to transition from the laboratory to the field by addressing two broad issue areas: the feasibility of introducing bacteria to mosquitoes in the field using safe "semi-field" conditions, and regulatory, ethical and social issues critical to the overall strategy.

Summary and significance

This new approach to fight malaria has already shown promising results in mosquitoes and triatomines. The strategy is compatible with existing control measures, including insecticides and insecticide-treated bednets, and is not affected by mosquito population structure. The prospects for implementation of this strategy in the relatively near future are more favourable than the prospect of transgenic mosquitoes. The entire approach is low-tech, since growing large numbers of bacteria is simple compared with rearing millions of male transgenic mosquitoes. This new approach's feasibility can be assessed in a relatively short time.

8.5 Population replacement strategies for dengue virus vector control *(Aedes)*

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Introduction

Population replacement refers to vector control strategies in which populations (or species) of mosquitoes capable of transmitting pathogens are replaced by those that cannot.[30] As envisioned originally, several approaches could be used to achieve this end, and recent work has focused on testing strategies based on linking genes conferring pathogen resistance (effector genes) to systems designed to spread the desirable traits through the vector population.[31, 32] Work to develop these strategies with Culicine mosquitoes (specifically, species within *Aedes* and *Culex*) is motivated by the need to control the transmission of arboviruses and filarial worms. A framework for testing population replacement was proposed that defines the context in which experiments are designed and establishes parameters by which success is measured.[33, 34] Three research areas were recognized, the first of which addresses the need to demonstrate in the laboratory that it is possible to engineer genetic resistance to pathogens in mosquitoes. The goals of this area are to discover and develop the expressed portions of effector genes that target specific infectious agents, identify functional control DNA fragments that direct their stage-, tissue- and sex-specific expression, and develop transgenesis so the effector genes could be integrated stably into the genome of the target species. The second area of research emphasizes the development of means for moving laboratory-tested effector genes into wild populations and requires the adaptation or invention of genetic mechanisms, defined collectively as gene drive systems, to increase the frequency of specific genes in those populations within an epidemiologically relevant timeframe. Furthermore, the efficiency of the gene drive system must offset the impact of introduced genes on the fitness of the transgenic mosquito and spread despite any load associated with the genomic integration of novel DNA. The third area of research involves comprehensive investigation to define and characterize target vector populations. These efforts are essential to understanding the scope of the proposed intervention, optimizing development and deployment of application protocols, and adopting and testing end-point parameters for success. The ultimate measure of success of a population replacement effort is a decrease or elimination of human morbidity and mortality due to the altered vectorial capacity of the vector.

Current status

Excellent progress has been made in the laboratory development of virus-resistant *Aedes aegypti*. Effector portions of genes based on RNAi or ribozymes can disable dengue viruses, resulting in reduced mean intensities of infection and prevalence[35] (also MJ Fraser, personal communication). In addition, single-chain antibodies targeting a conserved epitope of dengue envelope proteins have promise for preventing virus entry into insect tissues (KE Olson, personal communication). Multiple antiviral effector approaches are expected to mitigate selection for resistance to any single mode of action of an engineered gene. The recent successes were made possible by the use of control DNA sequences from mosquito-derived genes that direct expression and localization of effector molecules in the midgut, hemolymph and salivary glands, and by the development of transposon-mediated transformation technologies[36, 37, 38] (also Mathur and James, unpublished). These approaches can be applied to target other arboviruses, including yellow fever, chikungunya

and West Nile, and transgenesis has been achieved with *Culex quinquefasctiatus*, *Ae. fluviatilis* and *Ae. albopictus*[39, 40] (also L Alphey, personal communication). No reports have yet been published that describe explicit efforts to develop effector molecules that target filarial pathogens.

Several fundamentally different genetic mechanisms are being researched for adaptation to gene drive systems for Culicine mosquitoes.[31, 32] Safety and efficacy requirements are addressed in the design of two-component genetic mechanisms: one part imposes a lethal outcome following its expression, another rescues that lethality. Systems based on under-dominance, killer/rescue gene combinations and *Medea* are being developed for *Ae. aegypti*[41] (B Hay, personal communication; L Alphey, personal communication). *Medea* holds the most immediate promise because proof-of-principle has been demonstrated in the vinegar fly, *Drosophila melanogaster*.[42] Linkage of the effector genes to the components of these systems fixes the gene in the target populations. An additional approach seeks to exploit the ability of Class II transposable elements to spread rapidly through populations by linking antipathogen effector genes to them.[38] Self-mobilizing transposons that excise and integrate autonomously must be carefully designed to ensure safe and efficient spread through target populations while minimizing non-target organisms' risk of impact.[31] While it has proven to be straightforward to design and introduce engineered autonomous constructs into mosquito genomes, no unequivocal data exist demonstrating that the inserted DNA is able to remobilize.[43, 44]

Characterization of mosquito populations in disease-endemic regions is in progress. Target locales vary from densely populated urban areas to low-density rural areas, and the specifics of mosquito bionomics need to be determined before it is possible to predict the likelihood of success of a population replacement intervention. Research on dispersion, mating competitiveness, population structure, seasonal and spatial density, oviposition behaviour and insecticide resistance is expected to benefit population replacement strategies. The results of this work provide information on how far introduced mosquitoes might move and what the selective advantages or disadvantages of carrying transgenes are, as well as the extents of local demes, how many mosquitoes are in target areas, where the sub-adult stages are, and what the frequencies of insecticide-resistant genes are in target areas. All of these data are important when planning introgression of effector genes into local populations.

An important contribution to all phases of this work is the development of mathematical models that allow the comparison of different gene drive systems for the impact of fitness costs of the transgenes on gene spread and fixation, optimal release ratios, drive strength, environmental conditions, competition and other factors.[32, 45, 46, 47] This modelling permits critical decisions about which systems are more likely to have an impact on pathogen transmission and aids in experiment design and establishment of end-point parameters for determining success. Other efforts in support of population replacement strategies include a document that provides guidance for contained field-testing of mosquitoes carrying active gene drive systems.[48] This report outlines key issues associated with containment, regulation and experimental design for testing the safety and efficacy of gene drive systems, and makes recommendations

on phased testing, risk assessment practices and oversight responsibilities. Detailed protocols are yet to be developed for specific test situations based on the topics in the guidelines.

Issues

The issues listed here represent technical activities for which specific solutions are needed. Proof-of-principle must be demonstrated for a mosquito-adapted, gene-drive system and mechanisms for recall developed and tested. The latter will be useful to re-cycle the system with new effector genes or to remove those that are no longer needed or not effective.[42] Effector genes are needed that achieve near-zero prevalence of pathogens in transgenic mosquitoes. This may require the use of combinations of effector genes, especially to prevent emergence of resistant pathogens. An empirical definition of a pathogen-resistant mosquito must be established that reflects the true transmission potential (vectorial capacity). A reliable vertebrate animal model for transmission would be an asset in this regard. Additional field research is needed to understand vertical transmission and its potential impact on epidemiology. The local significance of sylvatic and zoonotic cycles of transmission also must be known, and addressed if necessary. More work is required on basic biology, most notably on mating behaviour and population structure of the target mosquito species to model the potential of population replacement.

Further refinement of molecular genetic technology will address some remaining challenges. Improvements in transgenesis and gene-targeting are needed to make them more efficient and mitigate issues with position effects and epigenetic gene silencing. We need to understand what is required to stabilize the expression of effector genes so that they remain effective throughout the control programme, and this may be facilitated by transgenic lines receptive to site-specific integration.[49]

Long-term storage of transgenic lines remains problematic. Some of the broader issues shared with research on Anopheline mosquitoes include the need for the sequencing additional genomes (in this case, *Ae. albopictus*), and more genomics-based work on gene expression (including developmentally regulated gene expression with respect to sex-, tissue- and stage-specificity and responses to pathogen infection) to support the identification of target genes and novel intervention approaches. Additionally, it would be helpful to have realistic methods for comparative studies and information transfer from other insects to mosquitoes.

Challenges

These challenges elaborate aspects of population replacement approaches that are wider in scope and where issues are unknown or remain to be defined. Social and political barriers to the use of genetically engineered insects must be identified, and where appropriate steps should be taken to offer specific solutions. Practical aspects include implementation of contained and open-field trials and identification of the necessary regulatory requirements and community engagement activities needed for the trials. The immediate and long-term successes of these efforts depend on awareness and discussion of their benefits. Therefore, communication channels among scientists, institutional administrators, government agencies and the public need to be established and maintained. Risk assessment procedures need codifying, as do concrete performance measures and evaluation parameters.

For longer-range decisions about development and deployment of population replacement methods, data-gathering methods are needed for accurate estimates of disease costs (including surveillance, treatment, prevention and categories of lost revenue such as tourism) in different endemic areas. Robust data analysis models are needed for cost-benefit projections of various control strategies. Scale-up plans should track development from small testing to manufacturing levels and incorporate good manufacturing procedures.

Additional needs

Despite increased interest in population replacement strategies,[50] more specialists are needed in all aspects of this work. This is true particularly for scientists from disease endemic countries. Established scientists, postdoctoral fellows and graduate students are needed with expertise in basic and applied sciences and in disciplines that can contribute to the cultural, ethical and social contexts of using genetically engineered organisms for disease control. Training opportunities, including short- and long-term courses, workshops and meetings, are needed for junior researchers and more established scientists contemplating moving into the field. The need is urgent for establishment of field sites for testing transgenic mosquitoes. These field sites need to reflect the diversity of transmission areas that make up the range of endemicity of arboviruses, and must involve facilities that can monitor the control efforts' impacts on disease epidemiology. Finally, it is essential that funding agencies continue to support basic science (discovery) while promising products are moved to development.

Recommendations

General recommendations include planning activities to envision how population replacement strategies will be integrated into control programmes that include other infection-suppressing and disease control measures to achieve maximum cost-effectiveness. Arguments have been made that integration should expand from considering only vector control efforts to include the impact of clinical interventions (for example, vaccines and drugs) and public health interventions that can alter dengue transmission.[51] Sufficient forethought will help research modules (laboratory, field, regulatory, etc.) work towards common goals optimized by mutual input. This broader thinking and planning is essential to have a sustained impact on disease prevalence. Consistent with this, vector control efforts are multidisciplinary, and earnest efforts should be made to maintain the pipelines of activities that allow virologists, molecular biologists, field ecologists, ethicists, regulatory experts and others to contribute their expertise. This is especially important as population replacement products move from the laboratory to field testing. More specific recommendations include the research topics addressed above. Guidelines for transport, testing and use of genetically-engineered vectors also must be standardized and meet the concerns of stakeholders. The WHO-TDR and FNIH technical consultation (of which this report is part) is a move in the right direction for this need.

8.6 Update on genetic approaches to population suppression and gene drive in *Anopheles*

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Several different genetic approaches to *Anopheles* control are being developed. These include approaches that are:

- **self-limiting** – repeated or recurrent releases are necessary to maintain the genetic construct in the target population. To have a significant epidemiological effect it will usually be necessary to release relatively large numbers of mosquitoes (inundative releases);
- **self-sustaining** – releases need occur only once or a few times, and the construct will increase in frequency of its own accord and maintain itself at high frequency. Releases can often be of relatively fewer mosquitoes (inoculative releases).

Two self-limiting strategies are currently under development in *Anopheles*. Release of **genetically sterile males** is the most risk-conservative approach, and is likely to be the first approach that is implemented in the field. Release of genetically sterile males can be epidemiologically effective under some conditions in reducing malaria transmission, and can also give useful information on the dynamics of genetically engineered mosquitoes in nature. Recently, Windbichler et al.[52] reported that B2-PpoI-carrying males are effectively sterile. PpoI encodes an endonuclease that in *An. gambiae* specifically cleaves the rDNA repeat, which is restricted to the X chromosome. The B2 promoter ensures that the enzyme is only expressed in the testes, while the male is making sperm. Males are effectively sterile apparently because the endonuclease protein is incorporated into the sperm and cuts the maternally-derived X chromosome in the zygote. Embryo development stops before nuclear fusion. This strain is testing in enclosed populations.

Sex-ratio distortion involves release of males that are fertile but produce mostly sons (which don't contribute to disease transmission or to population productivity), which in turn also produce mostly sons, can also be an effective means for reducing malaria transmission. In their study on B2-PpoI-carrying males, Windbichler et al.[53] found that the PpoI cleaved not only the maternally derived X chromosome in the embryo, but also cleaved the male's own X chromosome in the testes. This resulted in over-transmission of the Y chromosome, which was detected in about 90 % of the embryos (rather than the normal Mendelian 50 %). As noted above, these males are sterile, but further modifications of the genetic construct (reducing expression and/or reducing protein stability) should give a strain with the desired properties: fertile and producing mostly sons.

Self-sustaining approaches require some form of gene drive, usually based on a deviation from Mendelian transmission. Two different forms of drive are currently being developed in *Anopheles*.

A **driving Y chromosome** could be used by itself to produce a male-biased sex ratio in a self-sustaining manner. Alternatively, a driving Y could be used in conjunction with a resistant X chromosome – release of both would lead first to the spread of the driving Y, and then to spread of the resistant X. Any transgene linked to the resistant locus (e.g., a gene that blocks parasite development in the mosquito) would then spread as well. The data from Windbichler et al.[52] showing over-transmission of the Y chromosome due to PpoI-mediated cleavage of the X chromosome suggests that a driving Y chromosome could be made by putting the B2-PpoI construct on the Y chromosome.

Homing endonuclease genes (HEGs) are drive systems that occur naturally in fungi and other microbes. They bias their own transmission to the next generation by encoding an endonuclease that specifically cleaves chromosomes where they are not present; they then get copied across to the cut chromosome as a by-product of the repair process. A homing-based gene drive system in mosquitoes could be used in several ways, including (i) knocking out female fertility genes, thereby imposing a genetic load on the population and reducing the numbers of mosquitoes; (ii) knocking out a gene needed for malaria transmission; or (iii) knocking in a gene that disrupts malaria transmission. Windbichler et al[52] put the SceI homing endonuclease system from yeast into *Anopheles* cells and embryos and observed homing.

8.7 Collaboration and communication strategies between researchers and potential user communities

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Current status of collaboration and communication strategies

Interactions among the multiple actors associated with surveillance and control of vector-borne diseases are key factors in such activities' success. The collaborative nature of these interactions and communication strategies regarding normal control programme components permits a broader understanding and/or acceptance of potential genetic strategies. The current status of collaboration and communication strategies for genetically modified mosquitoes can be viewed from three primary perspectives: the research community, public health systems, and local communities/civil authorities, each of these responding to historical and sociocultural factors which need to be addressed for appropriate engagement and eventual acceptance. Each of these components is summarized below:

Status from the research community perspective:

- Current research administration (basic or applied/strategic) does not include in most cases and most countries the end-users at any point in the process of defining priorities, relevance, support targets, proposal composition, need for outreach etc.
- Few if any research request for applications (RFAs) are based on in tegrated and transdisciplinary research approaches, which would permit a more balanced perspective on the methodologies and strategies.
- Biomedical research is primarily focused on knowledge generation and not on public health or health-related goals; hence "health" goal-oriented research is extremely scarce.
- Researchers in most biomedical disciplines have "tunnel vision" and do not venture outside their specialties, nor do they want to be "bothered" by having to modify language and concepts, or integrate with other disciplines.
- Multidisciplinary or transdisciplinary research is more complex and time-consuming, and it involves learning new skills, methods and conceptual bases.

Status from the health care system perspective:

- Primary health care systems are almost universally vertical in structure, which precludes non-technical consultation or perspectives, or the integration of other sectoral considerations (i.e. environment, governance).
- Public health systems in most developing (and probably developed) countries do not consult or communicate (dialogue) with the public regarding programme strategies, and are only peripherally interested in health promotion as a method to achieve public assistance for tasks defined by public health system decision-making.
- Public health system professionals are not trained in communication skills, community engagement or consensus-building, and these activities are generally viewed as secondary or insignificant for their tasks (which are based on administrative indicators and are not goal-oriented).
- Legal clarity is lacking between the mandate or provision of health care programmes (implemented by government institutions), evidence-based structure and components and their acceptance by the public; generally the public simply avoids or does not abide by institutional guidelines or instructions when it does not agree with them.
- Few health care managers in the developing world respect or perceive a need or for or the value of their country´s academic community.

This may be associated with lack of priority for science and technology (low GNP investment). Technical expertise is not considered essential for decision-making, and often true technical expertise is officially hidden or rejected in favour of "political" decisions without any evidence base or social value. Most decisions on health or science technology are NOT evidence-based in developing and some developed countries.

Status from the user community (population and civil authority) perspectives:

- User communities do not have the experience of dialogue with institutions and sectors; they are neither prepared nor have they been approached to develop the skills required.
- User communities, at least in developing countries, have many sources of public opinion and governance strategies (traditional, cultural-driven, globalized) implying heterogeneous approaches for traditional and non-traditional political decision-making.
- In developing countries where vector-borne disease (VBD) disability burden is the greatest, there is broad mistrust of government programmes, PHS programmes in particular, their goals and how they will impact on families' health status. This mistrust extends to both preventive programmes (where most VBD programmes are managed) and medical attention.
- Most communities are neither aware of global influences nor versed in dealing with them.
- Most civil authority is not prepared to manage health care issues, and health is generally not on these organizations' agendas.

Issues, challenges, additional needs

The issues, challenges and needs which arise from these perspectives in terms of collaboration (participation) and appropriate communication strategies, specifically as related to potential genetic strategies, can be summarized as follows:

- Populations lack awareness regarding the transmission dynamics of VBDs and strategies currently used to prevent them. Government programmes do not openly discuss the strategies, the rationales behind their choices, their efficacy or their impacts. Hence, discussion of transgenesis as an option implies informing and participatory analysis of existing programmes from a critical and rational viewpoint, a topic not normally accepted by PHS (i.e. community engagement is viewed as radical or conflictive by government personnel).
- Communities continue to "receive" products proposed or developed by technical-scientific development (national or global), and are not part of a prior discussion regarding what options should also be explored (i.e. early engagement and discussion of disease control from the community perspective).
- Engagement of communities by researchers on health-related projects is novel, and will require careful structuring and ethical guidelines, since these will affect (as in any intervention) the way communities interact with PHS. Researchers (except from within part of the social sciences) are generally not aware of the social sciences, and consider attending social components as "noise." Institutional acceptance of researchers collaborating with communities is essential for continuity and to avoid conflict for the population.
- Risk assessment continues to be a difficult issue due to lack of critical information. In the absence of clear data, what criteria should be used: biomedical, environmental, social? What role should the community play in risk assessment?
- Although it is clear that community engagement should occur at a very early stage, who should

have the role of conducting engagement, who should fund this and what controls are needed for the processes?

- What role should local communities play in overall regulatory procedures? Communities are distant bureaucratically from federal agencies dealing with regulatory issues. How should their concerns be included in the regulatory process (bridge the global to local gap)?
- Educational tools are lacking to appropriately inform individuals with different roles in civil society (population, decision-makers, etc). Appropriate information and communication strategies are lacking for the different communities engaged (local, decision-makers, regional, state, institutions, NGOs, federal authority, academicians, journalists).
- There is insufficient analysis of the efficiency and impact of strategies developed for VBD control and their integration. A more complete approach to integrated vector control would permit discussion and inclusion of new strategies such as transgenesis.

Recommendations

There are no quick or simple approaches to address these issues, and most are associated with current fundamental deficiencies in PHS or VBD prevention and control strategies or academic funding policies in countries, social issues dominating institutional and community interactions, and in-country interactions between the academic community and government institutions. Addressing these components will require systematic analysis and engagement with government institutions, and shifts in policies which currently dominate responses to disease control. Addressing the need to engage communities at different levels (regional, local) on a permanent and continuous basis will be an important goal, equally essential to attain appropriate broad discussion and acceptance at all levels and to involve all actors required for sustainable development and disease control.

8.8 Potential areas of collaboration and communication strategies between researchers and potential user community of GM mosquitoes

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Introduction

Mosquito-borne diseases are still a major human and animal health problem in many countries. Diseases transmitted by mosquitoes include malaria, filariasis, yellow fever, Japanese encephalitis and dengue fever. The presence of multidrug-resistant strains of the pathogen, failure to develop novel vaccines, environmental and health concerns about chemical control, and development of insecticide resistance in mosquitoes have made controlling the diseases harder. Through the knowledge of mosquito-pathogen relationships and mosquito molecular biology it is possible to produce mosquito strains that are incapable of transmitting various parasites or viruses. Transgenic strains of mosquitoes have been developed and evaluations of these to replace or suppress wild vector populations, reduce transmission and deliver public health gains are an imminent prospect. The transition of this approach from confined laboratory settings to open field trials in disease endemic countries (DECs) is a staged process to maximize likely epidemiologic benefits while minimizing potential implementation pitfalls. Unlike conventional approaches to vector control, application of GM mosquitoes will face contrasting expectations of multiple stakeholders, the management of which will prove critical to safeguard support and avoid antagonism so potential public health benefits can be evaluated. Inclusions of key stakeholders in decision-making processes, transfer of problem-ownership to DECs and increased support from the wider malaria research community are important prerequisites for success. Contemporary developments in modern biotechnology, in particular GM, require competencies beyond the field of biology. The future of transgenic mosquitoes will hinge on the ability to govern the process of their introduction in societies in which perceived risks may outweigh rational and responsible involvement. Such developments, though remarkably promising, have potential risks that are not clearly understood and present challenges to communication and outreach strategies among the user community. In this review, the issues, challenges and communication strategies that would enhance the GM insect application protocol for the user community is discussed.

Strategy's justification and approaches

In the absence of an explicit policy and biosafety frameworks for engaging in safe deployment of GMM, which limits research, investment and innovative competitiveness, it is paramount that the design of any communication and outreach strategy be sensitive to the issues raised above. To carry out such outreach involving GMM user communities, networks of scientists must be established to conduct training (capacity-building); to hold workshops, seminars and policy round-table discussions; to train mass media to ensure continuous and credible media coverage, and to establish information resource centres for data capture, information exchange and delivery. The communication and outreach channels should include delivery of information material and creating strategic alliances with key stakeholders such as mass media, policy-makers, community leaders, professional groups in science and academia, civil society and special-interest groups as well as government, public health, medical and national environment departments. Issues of transboundary movement, ethical, legal, social, environmental and potential health issues would have to be considered in a communication approach.

Communication strategy issues and challenges

The strategy should focus both on general awareness and communication to popularize the GM vector innovation. In developing a communication strategy, it is important to recognize that communication is a two-way process that involves the *sender* of messages and the *receiver* with exchange of meanings. The challenges for effective communication therefore include overcoming:

- different information needs for different GM vector user stakeholders or communities;
- different languages among user communities;
- perceived interests – "branding" of GM innovations;
- communication may not be considered a national priority;
- mindsets; "proponents versus opponents" of GM vector use.

Public and community perceptions

Many factors influence community attitudes towards safety issues, public perceptions of risks, reactions to potential risks and willingness to accept scientific uncertainty surrounding risks. The development of effective communications messages therefore requires in-depth knowledge of target audiences including their levels of awareness and understanding of safety issues; their attitudes towards general safety and innovation safety in particular; possible impacts of communications on behaviour; and appropriate channels for effective dissemination of messages. The messages must be packaged with the target audience in mind with respect to language, content, channel and mode of delivery. It is also important to find out what the public wants to know about the GM vector technology and develop messages to provide such information, not what one thinks they need to know.

Bridging gaps between science and community knowledge

To be effective, communications must explain and contextualize risk and benefit of a technology. In developing public communications, the strategy should translate scientific evidence into clear, accessible and meaningful messages, addressing the needs of the community. In addition to explaining risk-assessment findings, the communications must also, where required, provide clear advice to the community on the action required in the strategy and must set out clearly any actions recommended for governments and other stakeholders. This requires close collaboration between risk assessors and risk managers, taking into account their respective roles.

GM vector use communication issues

In communications about GM vector use, understanding the factors that influence information uptake and decision-making process is crucial. These factors may include but are not limited to socio-economic and political interests; moral, ethical and legal issues; scientific evidence; and health and environmental concerns. For instance, good health is a fundamental human right but poverty restricts the choices of many communities, especially in areas faced with infectious insect-borne diseases. To develop the right messages, these factors must be considered.

Inter-institutional information networks

The current situation in GM technology innovations information dissemination is skewed, with most of the GM information coming from mass media and interest groups (mainly anti-GMO groups), with very little from GM experts. Inter-institutional collaboration is also still weak on GM information dissemination initiatives, especially in Africa. The world has increasingly become networked and a lot of damage can be done in a short time if negative information is disseminated widely.

Building credibility and confidence

The GM vector use communication strategy should embrace trusted and credible communicators *(messengers)*, use credible information sources *(messages)*, and use effective communication tools/channels such as mass media. In building credibility and trust, it is important to note that expression of empathy and caring *(sharing concerns in the area of health problems)* plays the largest part compared to competency or even honesty of messengers. Building credibility and confidence in the information disseminated on GM vector technology can be achieved by engaging credible third-party institutions such as universities, national academies of sciences, faith based-organizations, Nobel Prize winners and respected regulatory agencies to reach a wide range of community stakeholders. Messages should be able to withstand the tests of accuracy, balanced information and timeliness.

Communication strategy channels

The GMM communication should be done during seminars, workshops, conferences and round-table panels of expert discussions targeting: scientists/academia; government officials from relevant ministries (public health and sanitation; national environmental organizations; ministry of environment; ministry of medical services etc); policy-makers, legislators; media specialists; civil society and interest groups; and community leaders.

Other channels of communication to be considered include publications, web sites and demonstrations in public exhibitions. For efficient delivery, capacity-building has to be integrated in the programme.

Conclusion

Advances in knowledge of molecular biology of mosquito vectors and mosquito-pathogen relationships allow development of transgenic mosquitoes that are refractory to pathogens. Although the stability of these transgenic mosquitoes in a caged population can be determined and established under laboratory conditions, such fitness of the transgenic mosquitoes is diminished compared to wild-type mosquitoes. Once such problems have been overcome, the safety and effects of releasing these transgenic mosquitoes into the environment need to be fully assessed. Before contemplating release of transgenic mosquitoes containing active transposable elements, one must be aware of the possibility of horizontal transmission of the transgene to non-target species. Public acceptance of the release of transgenic mosquitoes must also be considered. The design of an effective communication and outreach strategy would have to ensure that all concerns are addressed for safe release of GMM. The communication strategy will have to consider issues such as information packaging, community language barriers and other stakeholder issues that influence decision-making processes for public acceptance.

8.9 Formulating an ecological risk assessment

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Two contrasting approaches to ecological risk assessment of genetically engineered insects will be addressed in this session. These approaches may be useful models for some countries considering the introduction of GM mosquitoes, but other countries may find their circumstances to be different. Hence there is a need to consider the ecological risk assessment (ERA) problem from general principles.

The most challenging aspect of ecological risk assessment is the formulation of problems. This is more of an art than a science. For GM mosquitoes, the main issue has been identifying potential harms or adverse effects on which to focus the ERA. Without this focus, one is left with a rather unsatisfying effort of trying to assess some kind of "average" effect on "biodiversity," which even if it could be measured well is of questionable significance. In technical terms, this has been called an adverse effects assessment, a hazard assessment or a safety assessment.

Another challenging aspect of the problem is that almost no one likes mosquitoes. This creates a challenge because most people are willing simply to dismiss the possibility that harm could come from genetically changing or eliminating some of them. In addition, the diseases that they transmit are so devastating to human populations that we often simplify the problem to either them or us. But ecological systems are not Manichean. Species are not either good or bad; they are some blend, even when they are mosquitoes. In some ways, if we can address ecological risks of mosquitoes in the deft way demanded by ecological systems, then we will have a model for much less demanding introductions, be they exotic species or some other genetically engineered animal.

There are no easy suggestions for how to solve this issue. The goal of the effort should be to identify possible risk hypotheses. A risk hypothesis is a causal chain connecting a stressor to an endpoint. Here the stressor includes the processes involved in producing and releasing GM mosquitoes as well as the released mosquito itself. In other words, concomitant alterations of infrastructure and management should be considered in addition to the mosquito as a possible source of harm.

An assessment endpoint is a technical term, but to oversimplify a bit, it can be thought of as the measure of the bad thing that could happen. Change per se is not bad. It is bad if the change runs against a social or cultural norm, or because a large group of people agree that it is bad. "Bad" is a reflection of human values. Consequently, in controversial cases these values should be made clear, and the people who hew to them should be identified. As an aside, it is possible to introduce notions of environmental justice at this point in the risk assessment process.

For GM mosquitoes, there is one guidepost by which to organize the search for risk hypotheses. The kinds of possible adverse effects are known. These are adverse effects on biological diversity, adverse consequences of gene flow, adverse evolutionary changes and adverse environmentally mediated effects on human health. However useful this has been, it may not be that enlightening if we get stuck simply repeating the category without being able to progress to specifics.

There are several ways to move to the specifics. Hayes et al.[54] illustrate a hierarchical holographic model. I will discuss two simpler approaches. The first is fault tree analysis[55] Fault tree analysis starts by listing all possible harms, whether they are caused by the stressor or not. The exercise is to work backward up the causal chain from the harm, and at each step enquire if it is possible to connect to the stressor. Fault tree analysis comes

hand-in-hand with event tree analysis, which starts with the stressor and tries to connect causal chains to a harm of any kind.[56] Most of the published enquiry on the ecological risks of GM mosquitoes has implicitly taken the event tree approach.

To conduct a fault tree analysis, harms must be identified first. Sometimes we may lack the originality to imagine harms, and sometimes our imagination is overly fertile and there are too many to address. There are many ways to address the second possibility using transparent, expert-driven, qualitative prioritization processes to limit the numbers. For GM mosquitoes, the first situation is more likely.

Another way to move to specifics is through a stakeholder process. A key element in stakeholder processes is identifying and engaging the proper stakeholders. I find the Mitchell et al.[57] typology of stakeholders to be quite useful for thinking about selecting stakeholders. Once a group of stakeholders is identified and engaged, they can be asked to describe their environmental values. Within these descriptions are characterizations of harm. Fortunately this kind of work has already been initiated for GM mosquitoes, although it is called an ethical or social issue at this meeting.

If we can specify significant environmental harms, it will be possible to conduct fault tree analyses to generate possible risk hypotheses. The existence of a risk hypothesis does not imply that there is a significant causal pathway. Indeed, even a demonstrably significant pathway does not imply that a risk is unacceptable. Although some distance and many steps separate the risk hypothesis and a decision, without clear, specific risk hypotheses a risk assessment is no better than watching leaves blow in the wind.

8.10 A precedent for genetic engineering: the environmental impact statement on transgenic fruit flies and cotton pink bollworm

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Use of genetically engineered fruit fly and pink bollworm in APHIS plant pest control programs is the title of the world's first environmental impact statement (EIS) on any kind of transgenic organism, either plant or animal, prokaryote or eukaryote. This programmatic EIS is also a major part of the world's first official government regulatory process on any transgenic insect. It was published October 2008 and is on the Internet at **http://www.aphis.usda.gov/plant_health/ea/geneng.shtml**. It was published by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). Two complete environmental assessments (EAs) on transgenic pink bollworms preceded this EIS.

This EIS is of major value for genetic markers and *Aedes*, possibly *Anopheles*, sterile insect technique (SIT) population suppression using repressible lethal genetic constructs instead of radiation to sterilize the insects. This EIS also has some applicability for population replacement strategies for *Aedes* spp. or *Anopheles* spp. using gene introgression/driver mechanism strategies.

When planning an environmental risk assessment for transgenic mosquitoes, it is imperative to determine what laws, as well as guidelines and regulations, apply in each country. Following applicable laws and regulations avoids prosecution and penalties, as well as court injunctions and lawsuits, whether frivolous or not. In the USA, either an EIS or EA may be required by the National Environmental Policy Act of 1969 (NEPA; *42 U.S.C. 4321 et seq*). The three species of fruit flies and pink bollworm are plant or crop pests and fall under the jurisdiction of the Plant Protection Act of 2000. Mosquitoes that bite livestock as well as humans are under of jurisdiction of the Animal Health Protection Act of 2002.

NEPA was made law to ensure that the environmental impacts of any federal or federally funded action are available to public officials and citizens before decisions are made and actions taken. NEPA applies within the USA and elsewhere in the world. Similar laws are now enacted in over 100 countries.

The EIS is a public and transparent process with stakeholder participation at the following phases:

1. At publication of Notice of Intent to do an EIS;
2. At scoping, several public meetings are held to plan the EIS extent and schedule;
3. At information gathering and consultation in drafting the EIS;
4. At the notice of availability of the draft EIS for public comment;

Other public stakeholder comments may be received and considered:

5. At the notice of availability of the final EIS with public comments and analysis of all relevant comments; and
6. At the publication of the agency Record of Decision, which may be the government's decision to implement the preferred or proposed alternative or not to implement it.

NEPA prescribes the following basic EIS components:

- Statement of Purpose and Need;
- The alternatives, including the No Action and Proposed Alternative;
- Description of the Affected Environment;
- Environmental (RISKS) Effects/Impacts/Consequences of Each Alternative;
- Draft EIS, Final EIS, and Record of Decision.

The species of the first transgenic insect EIS are:

- Mexican fruit fly, *Anastrepha ludens*;
- Mediterranean fruit fly, *Ceratitis capitata*;
- Oriental fruit fly, *Bactrocera dorsalis*;
- Cotton pink bollworm, *Pectinophora gossypiella*.

The alternatives presented in the EIS are:
- No action; continue programmes as they are;
- Expansion of existing programmes; SIT and other methods, including pesticides;
- The preferred or proposed alternative of integration of genetically engineered insects into government control programmes.

The biotechnologies of the preferred alternative are:
- Mass-rearing of either males and females or only male fruit flies with a marker gene, and then sterilization by radiation before release;
- Genetically sterilized male-only fruit flies that have a marker gene and that compete more effectively for mates than radiation-sterilized fruit flies;
- Fruit flies that produce only male offspring, which carry a repressible heritable sterility trait resulting in only males that carry the trait and no female offspring in the field;
- Mass-rearing of male and female pink bollworms with a marker gene, which are sterilized by radiation before field release (this is the only GM insect technology currently in large-scale, open-field trials);
- Mass-rearing of male and female pink bollworms that are genetically sterile without radiation exposure, producing males that are more competitive in mating with wild female bollworms than radiation-sterilized males.

Important appendices referenced in the body of the EIS are:
- Appendix B. Cooperation, Review and Consultation;
- Appendix C. Analysis of Repressible Lethal and Marker Genetic Engineering (biotechnology description);
- Appendix D. Risk Assessment Criteria and Analysis (Appendix D was modified after IAEA-TECDOC-1483, 2006, *Proceedings of a technical meeting 8–12 April 2002, http://www-pub.iaea.org/MTCD/publications/ResultsPage.asp*);
- Appendix E. Summary of public comments on the draft EIS;
- Appendix F. Analysis of impacts on endangered and threatened species in programme control areas.

This report addresses GM arthropods that affect both plants and human health. Only limited risk assessment criteria in the IAEA/FAO report were addressed as not all are applicable due to immobilization of the transposable element to mitigate horizontal gene transfer, biological containment (SIT function) and resulting short-term presence in the environment.

In regard to the phenotypes of GM organisms compared to the unmodified organism, many of the phenotypic characteristics considered to have hazard in the IAEA/FAO report are contingent upon some increased biological fitness or horizontal gene transfer (flow) occurring. However, for the GM-sterile insects of the EIS, biological fitness approaches 0 % for sterile males or sterile males plus females. For a heritable female lethal system, biological fitness would approach 50 % for the first generation of a female lethal system in which all females die but not males that carry the female lethal gene, but it approaches 0 % as females disappear.

Important biological fitness factors become biological performance factors in SIT applications for the following reasons:

- improved suitability for mass-rearing in large-scale production facilities;
- improved mating effectiveness, competitiveness and longevity over use of radiation;
- stability of the repressible lethal and marker constructs over multiple generations of mass-rearing.

In regard to horizontal gene flow in the EIS, exchange of genetic material between insects of different species and between insects and other organisms may be possible over evolutionary time, but it is not practical to quantify in the laboratory. The transposable elements (*piggyBac*) are removed or deactivated in the biotechnology addressed by the EIS to insure against horizontal gene transfer.

The EIS risk assessment conclusions are that the environmental consequences of the preferred alternative were found to have no more adverse environmental impact than the continuation or expansion of present SIT fruit fly and pink bollworm control programmes, which use radiation to sterilize insects and include other control and monitoring measures.

The Record of Decision (ROD) for the final EIS was published in the *USA Federal Register* (7 May 2009, Vol. 74, No. 87 pp 21314–16). This ROD authorizes the development and use of genetically engineered insects in sterile insect technique applications for USDA APHIS plant pest control programmes. The ROD states that the alternative that involves integration of genetically engineered insects into programmes is also the environmentally preferred alternative because the potential environmental impacts of this alternative are minimized by the resulting programme improvements and reduced to the extent that genetically engineered insects are incorporated.

8.11 Malaysia's regulatory experiences with regard to GM mosquitoes

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Vector-borne diseases such as malaria and dengue constitute a major obstacle to socio-economic development in much of the tropics and remain high on the list of priorities for the improvement of public health in Malaysia. Unlike other infectious diseases, vector-borne diseases are distinct because of their method of transmission that requires passage from human to human or animal to human via an arthropod vector. This transmission mode allows for the elimination of the disease through endeavours of removing the vector. Malaysia, specifically the Institute of Medical Research (IMR), is collaborating with Oxitec Limited from the United Kingdom to lower the incidence of dengue fever by reducing number of *Aedes aegypti* mosquitoes, the vector responsible for the disease.

The Malaysian Biosafety Act, although passed in the Parliament in 2007, has not been enforced yet. However, the Genetic Modification Advisory Committee (GMAC) of Malaysia acts administratively and undertakes risk assessments of all applications that are submitted to the Ministry of Natural Resources and Environment (NRE). The Institutional Biosafety Committee of IMR had submitted a brief of the project to NRE. The first phase is the containment study. GMAC undertook a stepwise risk assessment process of this collaborative project. The committee studied the technology to be used to reduce incidence of dengue fever. Using existing guidelines available to the committee, some of the factors taken into consideration were:

i) Purpose and description of the transformation methods used to develop the living modified (LM) mosquito;
ii) Suitability and type of the containment facilities used to test this technology (cage environment and room studies);
iii) Risk management strategies that were in place in the institute, and also the emergency contingency measures to mitigate potential adverse conditions; and
iv) Proper training of relevant personnel in arthropod handling procedures and biosafety guidelines.

Under the Malaysian Biosafety Act of 2007, the procedures followed from containment to restricted/open release will involve application to the National Biosafety Board (NBB) for approval before any release can take place. This application will be assessed using scientific risk assessment methods by the GMAC; relevant government agencies and the public will have a chance to submit their comments as well. Appropriate support from the community and relevant state authorities need to be obtained for the restricted trial. The suitability of the test site will be assessed and ecological monitoring will be carried out. Clear scientific proof of safety for humans and the environment and of efficacy are required. Regulatory authorities will assess the application to ensure that potential impact of the release is maximized while minimizing potential risks and other effects. The applicant will undertake monitoring and safeguarding the environment at the release site. Methods for environmental monitoring and providing the basis for collection of data will also be considered.

8.12 Thoughts on relationships with host communities for caged and open field release trials

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A central theme in the history of research involving human subjects is the concern that researchers will enlist or use individuals to answer scientific questions without adequate safeguards to ensure that their participation is both voluntary and reasonable in terms of any potential harms or benefits. Although common in the history of anthropology and other social sciences, the concept of community engagement has been slow to find traction in the biomedical sciences. But as advances in genetic technologies made it possible, and increasingly efficient, to characterize whole subsets of a population as "at risk" for various types of cancer, or to be a particularly important reservoir for infectious disease, these communities have suffered very focused discrimination and stigma. The biomedical research community has been forced to rethink the ethical and social significance of interacting with communities in research.

Guidelines for the ethical conduct of research are evolving and have begun to reflect greater attention to the interests of communities and the corollary obligations of investigators and research sponsors. But even with this increasing interest, we have not yet developed adequate accounts of which community engagement practices are necessary, under what circumstances and why.

Genetic and biologically modified mosquitoes raise a wide range of concerns in communities in which caged field trials or open-release trials are being conducted or contemplated. At a fundamental level, it is not clear even what precise harms might befall communities as a result of hosting such trials. Similarly, although we have well-honed (though often questionably effective) procedures to limit harm for individuals engaged in research (e.g. informed consent), we are at the very early stages of determining what procedures might be most useful, effective and appropriate for protecting communities participating in these trials, or even for gauging their willingness to participate in the first place.

My comments at the meeting will focus largely on our growing experience with community engagement in a wide variety of research contexts around the world and some preliminary thoughts about what might constitute effective community engagement in research involving modified mosquitoes. An initial framework for community engagement that was developed and proposed as a starting place for community engagement activities related to caged field trials of GM mosquitoes in southern Mexico has informed the continued evolution of the framework.

The key dimensions of the framework are (a) an explicit set of procedures or operating principles that provide a general architecture for how community engagement might be approached in practice and (b) an explicit set of ethical commitments or principles that provide the underlying rationale for why various approaches might be considered good or effective.

The framework itself is not the end product. Instead, it serves as an evolving "theory" of the effectiveness of community engagement and provides a convenient platform for collecting and analysing a wide range of empirical insights from a range of research contexts, not limited to GM mosquitoes.

A key interest for our research group at the moment is how we might build partnerships to allow our emerging framework to serve as a "collaboratory," an open-source, interactive resource that will allow the ongoing consolidation of insights about community engagement in global health in a way that will permit progress in our understanding.

Our ultimate aim is to develop an approach to community engagement that is equally valuable to investigators and communities and that lays out a shared set of commitments that can then serve as the basis for negotiations and specific decisions about initiating trials.

8.13 The importance of social research for public engagement in bio-control releases: The case of the Eliminate Dengue project

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Our central purpose is the development of more ethical, effective, stakeholder-directed and context-sensitive engagement strategies in Australia and Viet Nam. To achieve this, we use anthropology's proven systematic approach to social research to provide a platform for stakeholder engagement and draw on anthropological insights and research techniques to identify and develop solutions to issues that might impede the uptake of a biological initiative for dengue fever control (hereafter the *Wolbachia* method). At present, we are working closely with those likely to be affected by a *Wolbachia* intervention to negotiate, design and implement public engagement strategies in northern Australia and, from May 2009, in Viet Nam.

Why use an anthropological approach?

Anthropology's central contributions to sociological knowledge in the last century have been in establishing that all human knowledge is culturally and historically shaped, including people's understandings of disease, illness, cure and preventative measures.[58, 59, 60, 61, 62] Many commentators have argued that health interventions have been failing in part because they are based on a limited awareness of the complexity of local cultural contexts and the complexity of public interpretations and understandings.[61] This has led to a flurry of interest in anthropological methods in recent decades as these are noted for their sensitivity to context and rigorous examination of what people do, say and know, and the logics that underwrite these.[63]

Lay knowledge of biological control and genetic modification

Suarez et al.[64] argue that "…we still do not know what dengue is culturally and what it means for individuals in their everyday lives" (see also Slosek[65] and Gubler and Meltzer[66]). The same could also be said about biotechnology and genetic modification, which as concepts, practices and technologies are relative newcomers to the public domain and public consciousness. In Australia, past attempts to assess lay understandings of biological control interventions (including those using new technologies) have tended to focus on large-scale public opinion surveys. These often miss or barely scratch the surface of lay understandings, their history and the contexts in which they are generated. This is a real concern when decisions are being made on the basis of this research. It is especially crucial in the context of pest and disease management, where public knowledge and participation can be essential to the successful implementation of programmes and where public perceptions of biological control interventions can "play a crucial role in determining whether a particular technology is developed and adopted". [67, 68, 69]

Given this situation and the nature and complexity of the *Wolbachia* method, it is essential to gain a deeper understanding of stakeholders' knowledge about, for example, dengue fever, its vectors and transmission, understandings of bacteria, nature, biological control and genetic modification. Our Australian research strongly suggests that those likely to be affected by a *Wolbachia* strategy bring a range of knowledge and assumptions to their engagement with and comprehension of this method. While at times stakeholder understandings mirror biomedical or entomological knowledge, they often diverge from these in very particular ways. Indeed, we are encountering a range of different yet consistent understandings and perceptions of dengue, biological control, genetic modification

and bacteria in our research. This strongly suggests that local residents share certain assumptions about these issues that we can identify and address.

Central to this approach is the idea that without a clear understanding of such knowledge and the deeply held cultural, ecological or political assumptions that underwrites it, engagement strategies around new vector control methods will be less effective, less ethical and less authorizing at the stakeholder level than they could otherwise be. Unlike earlier studies, our approach includes a detailed, long-term and systematic investigation of these "public knowledges" and the taken-for-granted assumptions that underwrite them. The results of this research can greatly improve our capacity to: 1) communicate the nature of a *Wolbachia* intervention to a diverse population, 2) more fully comprehend stakeholder responses and 3) provide greater assurance to all parties that the public understand what it is that they are being asked to consider and evaluate. Thus, in our approach to community engagement we work to identify what people know and then use these insights to ensure that we are communicating with the public in ways that allow stakeholders to grasp what is being discussed and proposed, and what they are being asked to participate in and ultimately to agree to.

Deeper appreciation of lay knowledge opens up a space for the development of public engagement strategies that are potentially more ethical, nuanced, culturally sensitive and efficacious for informed decision-making by the public. This approach also has facilitated the following activities and outcomes at our Australian field site:

- Identify, inform and engage the multiple publics likely to be impacted by the *Wolbachia* method through interviews, focus groups and quantitative surveys and listen to their responses, questions and concerns;
- Examine the taken-for-granted (i.e. cultural) socially and historically constructed discursive practices and assumptions that underwrite these responses to improve our understanding of stakeholders' concerns and the socio-political setting in which we are working (i.e. the release site, the political and regulatory environment);
- Report these findings to stakeholders and the scientific team. Explore ways of responding to these issues, through education, the media, schools, new forms of participation and new scientific research aimed at exploring specific questions raised by the public;
- Explore and enact stakeholder-generated ideas regarding future engagement, communication, authorization and ownership.

8.14 Introduction to the ethical, legal and social implications of the potential use of genetically modified mosquitoes

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Introduction

The burden of malaria in developing countries with deficit of qualified and motivated human resources, lack of technological expertise and limited financial resources represents a major international challenge.[70] Various prevention and treatment strategies are being used to reduce malaria burden. They include intermittent preventive treatment for pregnant women and children, insecticide-treated nets, indoor residual spraying of insecticides and antimalarial combination therapies. Despite these proven strategies and the progress made, the elimination of malaria remains a major problem. The recent discussions related to the production and "authorization" of re-use of DDT in the fight against malaria vectors highlight the magnitude of the concern. Consequently, this brings consideration of using additional malaria control methods, such as genetically modified mosquitoes (GMM). Such a prospect, in addition to the complex technological aspects, brings implementation challenges to be addressed about ethical, legal and social implications, particularly in developing countries.

Ethical, legal and social issues

Culture, knowledge and religion influence ethical reasoning and decision-making

Ethics and the need for building capacity in disease endemic countries

"Tell it to me, I forget it. Show it to me, I remember it. Engage me, I understand it." (Chinese proverb)

There is a need for capacity-building (individual and community) and transfer of biotechnology in malaria-endemic countries to better address ethical, legal and social aspects of biotechnologies (e.g. genetically modified mosquitoes) for promoting engagement of individuals, communities and the public in the decision-making process about the development, potential use and evaluation of genetics-based methods for disease control.

Relativity of ethical practice

Ethical practice is relative, as cultural practice and religious tendencies influence ethical reasoning and decision-making. Learning about the realization and the limits of ethical standards within the context of different cultures, religions and nature-spiritual aspects may be a major discussion point between different stakeholders in the ethical framework of potential field release of genetically modified mosquitoes.

Information, communication, education and social mobilization

Information, communication and education are essential to implementation of new methods as they could facilitate community as well as public engagement. Individual consent doesn't mean community consultation, and vice versa.[71] In addition, the process must subsequently be accompanied by the development of a code of ethics based on a model of concerted decision-making. Steps and processes for communication at individual and community levels have to be developed from their domestic cultural framework and decision-making components, understanding and capabilities. This would allow actors to: (i) recognize the existence of an ethical paradox and dilemma, (ii) identify contentious points among the parties, (iii) examine the risks and advantages of each option, (iv) examine how traditional religions influence ethical reasoning, and (v) choose the most suitable solution and ensure each party involved is fully informed about the selected solution.

Important aspects to consider for the viability of such a system are the mechanisms and dynamics of decision-making at community level; these should be accompanied by means for information and training in ethics and by social/societal discussions about ethical issues.[72, 73]

Social and technological issues

These relate to the sustainability of researchers' financial resources and to populations in malaria-endemic developing countries in the framework of values shared and of direct and indirect advantages to be gained from the new biotechnologies. The insects' mobility gives significant potential for colonizing the environment, which raises the question of their obligatory and secured containment. This facilitates their study and solves the difficult question of controversy over the propagation of transgenic insects in the natural environment. Given the limited and sometimes contradictory data on the release of transgenic insects (genetically modified mosquitoes), the information available being mainly from experiments of short duration carried out in the USA,[74] it becomes crucial to ensure that regulatory agencies, ethics review committees and biosafety review boards are informed enough and receive the training necessary for them to undertake their tasks, particularly in developing countries.

Legal issues

Legal issues are often poorly addressed by national regulation in developing countries. In the absence of international recommendations, prospects for the use of genetically modified mosquitoes include several challenges to address. These include the issues of information, of education and of social implications for mobilizing the public, civil society and communities in malaria-endemic countries that already have some reservations regarding genetically modified organisms. Consequently, the approach to use needs to be based on the laws of biosecurity signatories of the Convention of Cartagena and of malaria-endemic countries, such as the 2008 Biosecurity Law of Mali. It can also be based on the experience of institutional and national ethics committees and on country regulations to initiate an integrated approach to address ethical issues (such as the Malian Empire's *Kurukan Fuga Chart*, 1235–1236[75]). Since the 20th century, developing societies have adapted and adopted (domesticated) a lot of scientific and technical (biological, mechanical, industrial and health, etc.) benefits at individual, community and public levels. This process is also useful for evolving developing societies' anticipated risk skills[76] and developing skills to understand the usefulness of food and health security. With these skills such countries will be able, beyond their *vulnerability*, to demonstrate responsible decision-making about many local, national and international questions by what Ajahn Virandhammo calls their decisional autonomy.[77]

8.15 Introduction to development of internationally acceptable guidance principles for testing and deployment of GM mosquitoes

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Genetically modified (GM) mosquitoes are being developed for use in vector control related to human diseases, such as malaria and dengue, under individual institutional or national guidelines on research and biosafety. Directed international guidance is lacking, however, on the development, testing and ultimate deployment of GM mosquitoes. Current uncertainty over which practices are widely acceptable may delay technical progress, deter investment in the technology and cause some national regulators to demand diverse and irrelevant data or to impose excessive safeguards. There is also a possibility that experimentation could proceed with inadequate safety in some countries with weaker regulatory systems that are under pressure to act in the face of rising impacts from disease. While we may look for lessons learned, guidance aimed at GM technologies in general – historically drafted in the context of GM crops intended for consumption – is not universally applicable to the particular technologies or circumstances of GM mosquitoes. The potential application of GM mosquito technologies across the wide range of disease endemic countries suggests that a broad consensus on the regulatory approaches taken at each stage of testing and deployment ultimately will reduce the burden on national resources and address concerns of cross-border effects from intentional release of living modified organisms. WHO/TDR has recognized this need and funds a project (MosqGuide, www.mosqguide.org.uk) to present best-practice guidance as a step in establishing this consensus.

MosqGuide has partners in Asia, Africa, Europe and Latin America working in consultation with national and international groups involved in technical development, testing and guidance on best practices or regulation. The project is designed in modules related to sequential stages of bringing GM mosquito technologies from laboratory to field. Module 1 provides an overview of current technologies and ethical, social and legal issues that must be addressed. Module 2 focuses on research and production issues. Module 3 concerns pre-deployment country decisions. Module 4 is about field release data handling and environmental monitoring. Module 5 comprises a pilot field study of public and regulator attitudes about alternative control methods, including GM technologies. Module 6 interfaces with capacity-building curricula. Finally, Module 7 is a prototype issues/response tool to support national or regional decision-making in disease-endemic areas regarding deployment of GM mosquitoes.

What guidance, by whom?

Users of GM mosquito technologies would benefit from guidance, guidelines or standards for the full range of activities needed to bring the technologies into use, such as laboratory practices and cage trials, site selection for field trials and first open releases, production and transport of GM mosquitoes and operational and environmental monitoring in deployment. Angulo & Gilna[78] suggest three objectives (in the context of self-dispersing GM insects): consistent problem definition, analysis and decision-making; minimal conditions for "wise use"; and compliance instruments.

Efficiency and understanding would be helped if common formats are adopted for risk analyses, and particularly for communication of risk regarding GM mosquito technologies. Such formats are agreed upon internationally in plant health, animal health and some areas of human health. Some detailed international standards exist for similar scenarios, such as release of biological control

agents and other beneficial organisms,[79] confined field release of GM insects,[80] and quality control and shipment of mass-reared sterile insects.[81] Although focusing on plant and animal pests, these provide useful cases of widely accepted current approaches to the issues and of models for the process of reaching agreement. The Organisation for Economic Cooperation and Development recently published a guide on preparation of consensus documents related to biotechnology.[82] A comprehensive set of internationally accepted biological information may be needed on particular species and technologies for GM mosquitoes.

Language to describe uncertainty and conclusions based on uncertainty has been carefully considered by the Intergovernmental Panel on Climate Change.[83] This covers sources of uncertainty, the use of expert judgement, the precision and calibration of terms, and the quantification of confidence and likelihoods. National frameworks also provide useful examples. The Australia/New Zealand Risk Management Standard 4360[84] is helpful in providing guidance on consistent qualitative descriptions of likelihoods and consequences in risk analysis. The New Zealand Environmental Risk Management Agency (www.ermanz.govt.nz) applies consistent procedures across the range of potential new organism introductions it judges. The Great Britain Non-Native Species Risk Analysis Panel (www.nonnativespecies.org) uses a model risk framework which translates into easily communicated graphics for managers on risk and uncertainty.

The complexity of risks potentially posed by GM mosquito technologies makes it difficult to put responsibility for guidance onto a single agency. Concerns include both environmental and health impacts and apply to both the Convention on Biological Diversity (CBD; www.cbd.int), particularly through the Cartegena Protocol on Biosafety (www.cbd.int/biosafety/), and to WHO (www.who.int). Intensive consideration by the CBD on the single issue of transboundary movement of GM organisms[85] indicated a need for broad coordination of many standard-setting agencies, and this is also likely in a comprehensive approach to guidance on GM mosquitoes. In the end, however, decisions regarding risks must be taken in the context of possible health benefits.

Estimates on the cost of preparing technical international standards in plant protection started at nearly US$ 200 000 for more conceptual standards,[86] with particularly controversial or complex standards costing up to US$ 3 million. A recent standard on a previously unaddressed issue (wood packaging material, ISPM 15) involved at least 1700 man-days of effort to reach agreement among the approximately 170 contracting parties. Attempts to agree on a common understanding of social responsibility have taken nearly a decade and will result next year in a guidance document (ISO/CD 26000) from the International Organisation for Standardization (www.iso.org), one of the few standard-setting bodies to require broader stakeholder inclusion.

Despite these costs, international guidance saves the burden of similar efforts at the level of individual countries while allowing adaptation to national realities. Implementation of international guidance can require significant national documentation in support of testing, production and deployment of GM mosquitoes. This need not be costly for the countries involved, however. Fees for approval of introductions of GM organisms into containment in NZ, which has a fairly vigorous regulatory cost

recovery policy, are approximately US$ 6500; fees for field testing are US$ 23 000 (current fees from ERMA New Zealand). The applicants' costs for documentation and hearings would be substantially greater than the regulatory fees.

Conclusion

The application of GM mosquito technologies would benefit from a comprehensive set of international guidance or standards. While there are precedents among current standards for similar processes, there are also divergent approaches which would need to be rationalized. The complexity of the issues involved suggests that guidance will need to be coordinated among a wide range of specialist technical and standard-setting agencies, rather than emanating from a single agency. This may add to the time and cost of guidance and present conceptual differences in approaches to risk, but it will save time and resources in the disease endemic countries where decisions on deployment ultimately will be made.

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Appendix 2 Example of a phased testing pathway for GMM

Efficacy and safety testing are envisioned to begin with small-scale laboratory studies, followed by testing in population cages in a laboratory setting conducted under appropriate containment guidelines. For promising strategies, the next phase may involve testing under semi-field conditions within a large outdoor cage that simulates the disease-endemic setting while minimizing the possibility for escape into the environment. Caged trials have been recommended before studies involving release,[1] although for specific technologies, given the proper regulatory, risk-assessment, monitoring and mitigating practices, these may be deemed unnecessary. Depending on results at these stages, subsequent testing would probably involve several phases of field release, beginning under conditions that limit spread into the environment (e.g. ecologically or geographically confined areas such as islands).

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Example of a phased testing pathway for modified mosquitoes

1 Laboratory studies

- Develop all components
- Characterize and validate designed functions of all components
- If applicable, generate transgenic mosquito lines and evaluate combined function
- Look for adverse target or non-target effects

2 Laboratory population cages

- Test efficacy and stability of the products in small- to medium size laboratory cages
- Evaluate fitness of transgenic infected mosquitoes
- Evaluate spread of target effects
- Look for adverse target or non-target effects
- Introgress effector functions into field-ready strains

3 Confined* field trials

- Evaluate fitness of transgenic mosquitoes under contained semi-field conditions
- Evaluate spread of target effects
- Look for adverse target or non-target effects and other safety requirements

*Physically contained (by cages); or biologically (unable to pass trait to subsequent generations) and/or ecologically confined so as to limit spread; select approach based on risk assessment and available containment and confinement options

4 Staged open field releases

- Continue ongoing safety assessment
- Evaluate persistence of the product
- Test mitigation plan
- Evaluate impact on entomological parameters
- If possible, evaluate direct impact on epidemiological parameters; if not, collect data for epidemiologic models to predict impact

Community engagement, Regulatory requirements, Communications

Appendix 3 Further reading

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DOI 10.2471/TDR.10.978-924-1599238

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ISBN 978 92 4 159923 8