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Deployment of Innovative Genetic Vector Control Strategies: Progress on Regulatory and Biosafety Aspects, Capacity Building and Development of Best-Practice Guidance

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Abstract. In the ongoing fight against vectors of human diseases, disease endemic countries (DECs) may soon benefit from innovative control strategies involving modified insect vectors. For instance, three promising methods (viz. RIDL® [Release of Insects with a Dominant Lethal], *Wolbachia* infection, and refractory mosquito technology) are being developed by researchers around the world to combat *Aedes aegypti*, the primary mosquito vector of viral fevers such as dengue (serotypes 1–4), chikungunya and yellow fever. Some of these techniques are already being extended to other vectors such as *Aedes albopictus* (the secondary vector of these diseases) and *Anopheles* mosquito species that transmit malaria. To enable DECs to take advantage of these promising methods, initiatives are underway that relate to biosafety, risk assessment and management, and ethical–social–cultural (ESC) aspects to consider prior to and during the possible deployment of these technologies as part of an integrated vector control programme. This is a brief overview of the objectives and timelines of some of the initiatives being championed by international institutions, including the United Nations Development Programme (UNDP), the World Health Organization (WHO) and the Grand Challenges in Global Health (GCGH) initiative co-sponsored by the Bill & Melinda Gates Foundation.

Keywords: Biosafety, Capacity building, Genetic Modification, Guidance, Insects, Regulation.

INTRODUCTION

Disease endemic countries (DECs) are showing interest in the possible benefits of innovative control methods involving modified (either as a genetic drive mechanism or through infection) insect vectors of human diseases. For instance, three promising methods Release of Insects carrying a Dominant Lethal gene (viz. RIDL, *Wolbachia,* and refractory mosquito technology) are being developed by researchers around the world to combat *Aedes aegypti,* the primary mosquito vector of viral fevers such as dengue (serotypes 1-4), chikungunya and yellow fever. Some of these techniques are already being extended to other vectors such as *Aedes albopictu*s (the secondary vector of these diseases) and *Anopheles* species that spread malaria. Therefore, these innovative strategies, and their agents, are coming to the attention of the regulators, vector control agencies, and policy-makers in DECs.

To enable DECs to take advantage of these promising methods and initiatives are underway that relate to biosafety, risk assessment and management, and ESC aspects

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to consider prior to and during the possible deployment of these technologies as part of an integrated vector control programme. Other initiatives which focused on agricultural applications also will prove useful background for DEC policy makers. This is a brief overview of the objectives and timelines of some of the initiatives being championed by international institutions, including the World Health Organization (WHO) and the Special Program for Research and Training in Neglected Tropical Diseases (TDR), the United Nations Development Programme (UNDP), and the Grand Challenges in Global Health (GCGH) initiative co-sponsored by the Bill & Melinda Gates Foundation.

The longest history for consideration of these issues for control of vectors of human disease are the TDR-led international expert consultations (WHO, 1991; Takken W & Scott T, 2002; Knols *et al.*, 2004; WHO, 2009a) as well as other fora, such as the Vector Biology Network (Beaty *et al.,* 2009). A review of the science and regulation of genetically modified (GM) insects in the USA was also conducted in 2004 by the Pew Initiative on Food and Biotechnology (Pew, 2004). Some initiatives focused primarily on the control of plant pests, such as the joint IAEA/FAO/IPPC technical meeting (IAEA, 2006), United States Environmental Impact Statement (EIS) and North American Plant Protection Organisation (NAPPO) standard, set a precedent that provides conclusions and models for approaches to these issues. The UNDP risk assessment workshop, the Pew Initiative and both of the plant protection initiatives mentioned above have been completed with final conclusions and documentation available. The rest of the initiatives reviewed here are expected to be concluded within 3 to 5 years. This article gives a brief overview of these initiatives, their objectives and timelines, which are summarised in Table 1.

The outcomes of these initiatives vary in their legal status, with some being legally binding in some way, some being guidelines, and some simply guidance documents for voluntary application. Guidelines are statutory documents, which often, but not always, overlap with legal requirements, e.g. WHO Guidelines which are then applied on a national level through policies, regulations or legislation. Guidance documents in this context are for voluntary application; when the source is well recognised, however, they are usually followed to achieve regulatory harmonisation and save resources that would be required to prepare frameworks on a country by country or ad hoc basis.

Promising innovative genetic vector control strategies. Strategies for controlling mosquito-borne diseases using modified mosquitoes can be broadly categorized based on two independent criteria. These are (i) intended phenotypic outcome and (ii) intended genotypic outcome in terms of persistence and spread or otherwise of the modification in the environment. Phenotypic outcome: conventional vector control practices such as source reduction and the use of insecticides generally aim to reduce transmission by reducing the overall number of (female) mosquitoes. This can also potentially be achieved by genetic methods; such approaches are known as 'population suppression' strategies. Alternatively, it may be possible to alter the mosquito population to a less harmful form, for example by making the mosquitoes unable to transmit specific pathogens. Such approaches, known as 'population replacement' strategies, have two essential steps. The first of these is to identify a heritable modification that will make the mosquitoes less harmful; the second is to introgress this modification into a wild mosquito population (Alphey *et al.,* 2002; Riehle *et al.*, 2003).

Persistence and spread of the modification: modifications may be self-limiting or self-sustaining in the target wild population. Self-limiting systems will, by design, be eliminated from the target population over time, e.g. by natural selection. The modification is then maintained in the wild population only by periodic release of additional modified mosquitoes. The speed of this elimination may vary from one strategy to another; for example a dominant lethal or sterilizing transgene will be completely eliminated in one generation, whereas a construct with a milder fitness penalty may persist for several generations. Nonetheless, self-limiting systems will neither persist indefinitely nor spread significantly beyond the target area. In contrast, self-sustaining systems are intended to persist indefinitely, and indeed to increase in prevalence, e.g. allele frequency, in the target area and beyond. These properties may make deployment of such systems relatively inexpensive, as they may be able to spread from a relatively small release. However, their indefinite presence in the environment, and potential to spread into new populations, may raise additional regulatory and social concerns (Angulo and Gilna, 2008a; Angulo and Gilna, 2008b).

Three specific examples of genetic control systems currently under development may help to illustrate these properties. These are RIDL (Release of Insects carrying a Dominant Lethal gene), *Wolbachia* (*w*Melpop) and MEDEA.

RIDL is a variant of the Sterile Insect Technique (SIT). SIT is an environmentally safe and species-specific method of pest control that depends on the release of large numbers of sterile insects into the target area (Dyck *et al.*, 2005; Knipling, 1955). These mate with the wild insects; progeny of such matings are non-viable and so the wild population tends to decline. If sufficient sterile insects can be released for a sufficient period the target wild population can be controlled or even eliminated by this method; large scale successes have been achieved against several major agricultural pest insects. Sterile Insect Technique (SIT) has been successfully applied for decades controlling agricultural insect pests (Robinson, 2002) and is emerging as a mosquito control technology (Ageep *et al*, 2009). RIDL applies recent advances in genetic engineering to offer solutions to some issues of using SIT in mosquito control programs, such as the separation of males and females and the need for sterilization by irradiation (Heinrich and Scott, 2000; Phuc *et al.*, 2007; Lee *et al.,* 2008, Khongtak *et al.*, 2009 Alphey *et al.*, 2009) RIDL is a self-limiting method for population sup-

pression, as are other proposed modifications of SIT using modified mosquitoes (e.g. Benedict and Robinson, 2003; Brelsfoard *et al*., 2008; Catteruccia *et al*., 2005).

Wolbachia are Rickettsia-like intracellular bacteria. They are transmitted from a mother to her progeny, and behave like a heritable genetic element (e.g. mitochondria) rather than an infectious agent. They spread themselves through wild populations by manipulating their host's reproduction so that they are inherited disproportionately. *w*Melpop is a pathogenic mutant of *Wolbachia*, originally identified in *Drosophila* but artificially transferred to *Aedes aegypti* (McMeniman *et al*., 2009). It shortens the adult lifespan of infected individuals, at least in the laboratory. In mathematical models of dengue, reducing the lifespan of adult females would significantly reduce transmission of the virus. wMelpop retains the key features of *Wolbachia* that allow it to invade and spread through target populations (Xi *et al*., 2005). *w*Melpop is therefore a self-sustaining population replacement strategy.

Infection by *w*Melpop represents a heritable modification, but not a product of recombinant DNA technology so not a GM mosquito. An effective method to drive transgenes in wild mosquito populations has yet to be demonstrated (Sinkins and Gould, 2006). One leading candidate is the use of synthetic MEDEA-like elements. MEDEA (Maternal Effect Dominant Embryonic Arrest) is a selfish DNA system discovered in the beetle *Tribolium castaneum* (Beeman *et al*., 1992). Like other selfish DNA systems, MEDEA elements can spread by non-Mendelian inheritance despite not conferring a direct fitness advantage to the individuals that carry them. A synthetic MEDEA-like element was constructed in *Drosophila* and shown to be able to spread within target laboratory populations (Chen *et al*., 2007). If implemented in a mosquito, and connected to a gene capable of preventing transmission of a pathogen, such a MEDEA-like system could form the basis of a self-sustaining population replacement strategy.

Despite much laboratory progress (e.g. Capurro *et al*., 2000; Franz *et al*., 2006; Sperança and Capurro, 2007); development of suitable effector genes is also at only proof-of-principle stage. Even when all the technological challenges are overcome, self-sustaining strategies will face biosafety, ethical, legal and social concerns related to the release of modifications intended to invade wild populations and to persist indefinitely in the environment. Suppression strategies using RIDL and similar self-limiting systems are considered to be of less risk compared to self-sustaining technologies. The RIDL trait will never be fixed in the wild population and any unanticipated effects can be reversed simply stopping releasing. In any case, it is important to emphasize that the possible risks associated with the release of genetically modified mosquitoes must be assessed on a case by case basis and balanced with potential benefits of reducing the incidence of diseases.

Relevant concepts of biosafety.

Biosafety, as a concept, is described in the Convention on Biological Diversity (CBD, 1993) as ensuring an adequate level of protection in the fields of safe transfer, handling and use of living modified organisms resulting from modern biotechnology, recognising that they may have some potential adverse effects on the conservation and sustainable use of biological diversity, or of human health. It can apply both in the laboratory and for human health and environment. It is fundamentally an issue of risk analysis, and so involves principles embodied in formal risk identification, assessment, management and communication. The outcome of biosafety risk assessments and management must be balanced against benefits derived from the expected efficacy of introducing new organisms or genetic modifications of organisms in benefit cost analysis processes that are also rigorously defined and which represent the range of dimensions of significant interest to society. This would include not only economic indicators, but the outcomes must also meet broad social, ethical and cultural criteria to ensure that performance is relevant, effective and efficient. The Grand Challenges in Global Health program have engaged ethicists and social scientists to look at ethical social and cultural outcomes within the Grand Challenges programs (Singer *et al*., 2007, Tindana *et al*., 2007), including the of the use of GM Vectors, of which some of the work has already been published (Lavery *et al*., 2008).

Many developing countries are now starting to develop their regulatory frameworks governing GMOs as a result of the implementation of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, commonly known as the Cartagena Protocol (or CPB). Where regulatory frameworks exist, they are unlikely to be specific to insects and the countries often do not have sufficient resources to enforce such legislation. Capacity will be therefore key in the areas of administration, legislation, science and enforcement and monitoring.

National Biosafety Frameworks. Using Brazil as an example of a national regulatory framework, the first National Legislation to regulate laboratory research and production of genetic manipulate organisms for health and agriculture, was approved in 1995 (Law 8.974). This law created the National Biosafety Technical Commission (CTNBio) which is part of the Ministry of Science and Technology, which is an advisory multidisciplinary collegiate, that provides technical and assistance support to the Federal Government to formulate, update and implement the National Biosafety Policy for GMOs and their by-products. It also establishes safety technical norms regarding the authorization of research-related activities and the commercial use of GMOs and their by-products, based on the evaluation of their zoophytosanitary, human health and environmental risk.

In December 15th 2003, the Brazilian Government approved the Law 10.814 specifically for the culture and commercialization of a GMO soybean crop. On March 24th 2005, Brazil approved Law 11.105 that regulates items II, IV and V of Paragraph 1 of Article 225 of the Federal Constitution, providing for safety norms and inspection

Table 1: Biosafety Initiatives, Timeline and Outputs for GM Insects.

Name and sponsorship of initiatives	Participants	Timeline	Outputs	Significance
WHO/TDR Technical Consultations	Global experts in insect molecular biology, vector biology etc.	1991 McArthur Foundation/TDR/University of Arizona meeting (reviewed in Beaty *et al.*, 2009) 2002 Frontis Workshop on Ecological Aspects of the Use of GM mosquitoes (Takken *et al.*, 2002) 2004 Bridging laboratory and field research of genetic control of disease vectors, Nairobi, Kenya (Knols *et al* ., 2004) 2009 FNIH/WHO Technical Consultation on GM Vectors (WHO, 2009a) Ongoing in 2010.	Global consensus about appropriate biosafety and biosecurity stages prior to deployment of modified vectors for disease control	Coordinated approach among researchers worldwide
International Atomic Energy Agency (IAEA), International Plant Protection Convention (IPPC) Secretariat and the Food and Agriculture Organisation (FAO)	Global experts in insect molecular biology, vector biology, regional and national governance and regulatory frameworks	Technical meeting to develop guidelines for risk assessment of transgenic arthropods held in 2002 (IAEA, 2006)	Compilation of case studies and conclusions from break out sessions on various aspects of GM arthropod research, testing and release	Guidance for risk assessment of transgenic arthropods for control of agricultural pests and human disease vectors
Pew Initiative on Science and Regulation of Genetically Modified Insects	Global experts in insect molecular biology, vector biology and USA regulatory frameworks	Concluded in 2004 (Pew, 2004)	Report on the status of scientific development and the appropriateness of the USA regulatory framework for genetically modified insects	Co-ordinated review of the status of science and regulation in the USA
Daegu Protocol	Global experts in insect molecular biology and vector biology	Presented at the Insect Biology and Industry Meeting, Korea, 2007	Advocates Environmental Impact assessment as an appropriate risk assessment tool for genetically modified insects	Consensus among researchers in the field
Environmental Impact Statement (EIS) on the Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs	USDA/APHIS with experts and public consultation	Completed May 2009; Record of Decision 7 May 2009 (Federal Register, 2009 (71 FR 21314-21316)	Decision making tool for incorporation of modified insects into existing Sterile Insect Technique programs	First national environmental risk assessment of GM insects for deployment in area-wide pest control programs

North American Plant Protection Organisation (NAPPO)	Expert Working Group under NAPPO (Mexico, USA and Canada)	NAPPO Standard RSPM 27 Completed Oct 2007 (NAPPO, 2007)	Regional standard for import, transport and confined field release of GM arthropods for plant pest control	Creation of a regional legal agreement on procedures for confined field release
United Nations Development Program and University of Malaya	Malaysian policy makers and scientists, international resource trainers	November 2008; (Beech *et a*l., 2009)	Capacity building for risk assessment of transgenic insects	Malaysia is one of the first countries to consider use of GM mosquitoes for disease control
Gates/ FNIH Core Working Group on Guidance for Contained Field Trials	GCGH recipients, GCGH program staff, ESC program members and expert scientists	Published Benedict *et al.* (2008)	Paper on Guidance for Contained Field trials of Vector Mosquitoes Engineered to contain a Gene Drive System: recommendations of a scientific working group	Recommendations for contained trials of modified mosquitoes containing a gene drive system.
MosqGuide	Experts from Imperial College London, Oxitec Ltd, University of Sao Paolo, University of Nairobi, Mahidol University Thailand, Gorgas Institute Panama, INSP Mexico	2008-2011: Modules 1 and 2 for consultation in 2009; Modules 3 and 4 for 2009/10; Modules 5 and 7 for 2010.Module 6 ongoing. (Mumford *et al.*, 2009)	Best practices for the use of GM mosquitoes for control of Dengue and Malaria in Disease Endemic Countries	Provide guidance in support of national decision making on use of GM mosquitoes
Ad Hoc Technical Expert Group on Risk Assessment and Risk Management, Cartagena Protocol	Members of Expert Working Group and resource people	2009-2011: draft guidance anticipated April 2010	Road map and Guidance on risk assessment and risk management for LMOs, in accordance with Annex III of the Cartagena Protocol	Specific guidance for risk assessment and management of GM mosquitoes
Biosafety Training Centres in Genetically Modified Vectors	University of Bamako, Mali, Africa; Centre for Medical Entomological Research Madurai India; Medellin, Colombia	2008-2011	Capacity building through regular regional training courses targeting researchers, policy makers, regulators, etc in regulatory frameworks, biosafety, risk assessment, and ESC for use of GM mosquitoes	The creation of networks of professionals trained for decision making and safe use of GM mosquitoes for vector control
WHO Epidemic and Pandemic Response Unit	Same as above	2008-2011	Capacity building in laboratory biosafety and biosecurity	A network of extensively trained laboratory experts who will train others in their regions
Ethical, Social and Cultural Program for the Grand Challenges in Global Health (GCGH) Initiative	Based in Toronto, with team members in India, Ghana and South Africa	Ongoing throughout the GCGH projects	Global case studies on community engagement; and guidance on site selection and establishment of caged field trial sites for modified vectors	Present options for decision making in ESC

mechanisms for activities that involve genetically modified organisms GMOs and their by-products, implements the National Biosafety Council (CNBS), re-structures the National Biosafety Technical Commission (CTNBIO), provides for the National Biosafety Policy (PNB). The publication of this Law revokes Law no 8.974, of 5 January 1995, and Provisional Measure no 2.191-9, of 23 August 2001, and arts. 5, 6, 7, 8, 9, 10 and 16 of Law no 10.814, of 15 December 2003, and provides for other measures (CNTBio, 2005). It also established the National Biosafety Council (CNBS) which is subject to the Office of the President of the Republic as a higher agency of the President of the Republic for formulating and implementing the PNB.

The new law was based on the UN Cartagena Protocol on Biosafety agreement that has been ratified by 156 countries known as Parties to the Protocol (CBD, 2009a), most of which are developing countries. This Law provides for safety norms and inspection mechanisms for the construction, culture, production, manipulation, transportation, transfer, import, export, storage, research, marketing, environmental release and discharge of genetically modified organisms – GMOs and their by-products, guided by the drive for attaining scientific development in the biosafety and biotechnology area, the protection of life and human beings, of animal and plant health, and the compliance with the principal of environmental precaution. Under this Law resolutions can be added on specific subjects that the Law does not currently cover. As it does not cover Genetically Modified Insects (GMIs) Biosafety Legislation is urgently needed to compensate for the deficiencies of the Law and Resolutions in this area. Due to interventions from researchers in the GM insect field a GMI specific Norm is currently in the drafting process.

A further example of existing regulatory frameworks adapting to the use of GM Insects is given by the preparation of an Environmental Impact Statement (EIS) by the USA Department of Agriculture (USDA). The EIS is required under the USA National Environmental Policy Act of 1969, 42 U.S.C. 4321 et seq. and its implementing regulations. This Law requires Federal agencies to do an assessment of the environmental effects of their proposed actions prior to making decisions. Two major purposes of the environmental review process are better-informed decisions and citizen involvement. The EIS conducted by the USDA chose the plant pests, Pink bollworm (Lepidoptera: Gelechiidae) and Tephritid fruit flies for use in USDA APHIS plant pest control programs. There had been laboratory studies and confined field tests to test the safety and efficacy of certain traits within these species, but the purpose of the EIS was to examine the environmental impact, against the existing alternatives, of the use of these strains in agency eradication actions or preventative release program strategies. A Federal Register notice (71 FR 75933-75934, Docket No. AHPIS-2006-0166), in Dec 2006 announced an intent to prepare the EIS, followed by the publication of the draft EIS in May 2008 (73 FR 3115) by the Environmental Protection Agency (EPA). The draft EIS was subject to a public comment period which was open until Aug 2008. As part of the public comment period 5 public meetings were held across the USA. In Oct 2008, the final EIS (USDA, 2008) was published in the Federal Register (73 FR 67511 Docket No. ER-FRL-8587-5) which included discussion of the seven public comments received on the draft EIS. A further public comment period was open until Dec 2008. A Record of Decision, was published in the Federal Register in May 2009 (74 FR 21314-21316), which indicated that of the alternatives investigated for environmental impact in the EIS, the use of genetically engineered insects in the plant pest control programmes was the environmentally preferred alternative and would be integrated by the programs. This represents the first systematic environmental risk assessment on GM insects and is the first one the USDA has completed on any genetically modified organism, to date. This recent decision to integrate the use of genetically sterile insects into the plant pest control programs provides an important precedent, where the EIS was the format used to meet governmental requirements and to provide a format for public comment and transparency in the decision making process. This mechanism was also suggested at the Regulatory Issues Session of the Insect Biotechnology and Industry (ICIBI2007) meeting held in Korea in 2007 (Daegu Protocol, 2007).

Regional activities for the regulation of GM insects. The North American Plant Protection Organization (NAPPO) is a regional Plant Protection Organization of the International Plant Protection Convention, coordinating the efforts among Canada, the United States and Mexico to protect their plant resources from the entry, establishment and spread of regulated plant pests, while facilitating intra/ interregional trade. In October 2007, NAPPO signed a new Standard, RSPM27 "Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries" (NAPPO, 2007). "Confined Field Release" is broadly defined as "Release of organisms into the environment under specific conditions and restrictions intended to prevent establishment in, or control the spread into the environment, and/or limit the unintended interactions with the environment, of the organisms and any progeny derived from them". Relevant confinement measures may "include the use of one or a combination of the following methods:

- o Physical confinement such as the use of arthropod proof caging…
- o Biological confinement such as the release of sterile transgenic arthropods…
- o Geographic isolation…"

Sterility may be by irradiation or by genetic methods. Geographic isolation means release of organisms outside their normal habitat or range so that they cannot establish. The Standard does not cover unconfined releases, i.e. releases where none of the above confinement measures apply, though aspects of it any be useful in such cases, and also for

arthropods which are not plant pests.

The Standard also covers importation into containment facilities (also known as quarantine facilities or laboratories). It is anticipated that this would be the first step even for insects intended for confined field release, but also that in many cases insects will be imported into containment facilities for research or other purposes with no intention of field release.

The standard was developed by an Expert Working Group (EWG) which first met in March 2006. This comprised representatives of NAPPO, of national scientific and regulatory agencies from the three NAPPO countries (Canada, USA and Mexico) and two international experts (NAPPO, 2006). After initial drafting by the EWG, the country representatives led a consultation period in each member country. This consultation led to further revision and a final version which was signed by the NAPPO Executive Committee. Each NAPPO member state commits itself to incorporate signed standards into its national legislation, or to align its existing regulations with the published standard and therefore the standard is legally binding in the NAPPO member countries.

International Activities on Guidance Documents and the Regulation of GM Insects

MosqGuide

The World Health Organisation Special Programme in Research and Training in Tropical Diseases (WHO/TDR) has funded a project, "Best Practice Guidance for Deployment of Genetic Control Methods against Mosquito Vectors in Disease Endemic Countries" designated MosqGuide, to develop guidance on the potential deployment of different types of genetically modified (GM) mosquitoes to control vector-borne diseases, specifically malaria and dengue. This guidance is intended to support disease endemic countries (DECs) and other stakeholders in considering the safety and legal/regulatory aspects, as well as ethical, cultural and social issues, of such deployment.

The MosqGuide project, led by the Centre for Environmental Policy at Imperial College London, has created a network of expertise in vector biology genetics, disease control, regulation, social science and risk analysis from the UK, Panama, Brazil, Mexico, Thailand, Kenya and India. The purpose of the network, which is funded by the WHO Special Program in Training and in Tropical Diseases (TDR) is to prepare guidance on best practices, peer reviewed literature, emerging data and related experiences of risk assessment and management. The project itself is not involved in any field release programmes, although partners may be under separate funding. The project was launched in July 2008 with a network meeting at Imperial College London where the parameters of the guidance were specified. The MosqGuide project will address issues surrounding the deployment of GM vectors where the mosquito deoxy-ribonucleic acid (DNA) has been directly modified, but it will not include other potential strategies to control mosquito vectors, such as paratransgenesis (Riehle *et al.*, 2003). The guidance will also concentrate efforts on addressing technologies likely to reach field use within ten years of the project start date (for implementation up to 2018). The guidance is being prepared as seven modules, each with a specific target audience, and drawing on expertise with risk benefit methodologies, consultation and experience to prepare and validate guidance documents relating to deployment of genetically modified mosquitoes to control the mosquito vectored diseases of dengue and malaria.

Cartagena Biosafety Protocol Initiatives. An initiative has recently been instigated under the Cartagena Biosafety Protocol, under the auspices of the Ad Hoc Technical Group on Risk Assessment and Risk Management (AHTEG) to gather information on risk assessment and risk management for living modified mosquitoes (LMM) in accordance with the general principles of scientifically sound risk assessment under Article 15 (Annex III) of the Biosafety Protocol (CBD, 2009b). These include the following concepts: Risk assessment should be carried out in a scientifically sound and transparent manner; Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk; Risks should be considered in the context of risks posed by the non-modified recipients or parental organisms; and that Risks should be assessed on a case-by-case basis. Further information on this initiative is available on the Biosafety Protocol Website as well as a further note in this journal. The sub-working group on LM Mosquitoes was formed to produce modalities for development of guidance documents on risk assessment and risk management. In the context of the steps contained in paragraph 8 of Annex III of the Protocol, the general structure of these guidance documents should be organized by providing: (i) points to consider; (ii) rationales for the points to consider; and (iii) relevant bibliographies and supporting documents. An open-ended online forum has recently closed where participants were involved in addressing the points above. The outcomes of the sub-working group will serve as basis for an informed discussion during the regional real-time online conferences (tentatively scheduled to take place in February 2010). Further details are given in the report of the meeting (CBD, 2009b).

Capacity building. WHO/TDR-funded Regional Biosafety Training Centres. In response to the need for capacity strengthening in developing countries regarding the deployment of genetically modified vectors, WHO/TDR has recently funded the establishment of the Regional Training Centres in Africa, Asia and Latin America (WHO/TDR, 2009b, 2009c). The main goal is to train and prepare a pool of public health workers and researchers working in the fields related to genetically modified vectors to acquire knowledge and experience necessary for the application of biosafety and regulatory principles and practices. In particular, the specific objective is to strengthen their capacities for assessment and management of the potential risks of hu-

mans and the environment of the use of genetically modified vectors. The biosafety training courses have taken place or are expected to be organized in Mali (Africa), India (Asia) and Colombia (Latin America) each year. These courses are offered simultaneously with the laboratory biosafety and biosecurity training courses organized by the WHO/TDR staff. In general, 10-15 participants in relevant fields will be selected in each region to attend the training courses each year. A coordination committee is established to standardize the course contents across the three Regional Training Centres and link with the MosqGuide project. The course contents are focused on knowledge regarding genetically modified vector technologies as well as regulatory process, risk assessment and ethical-social issues related to best practice in the deployment of genetically modified vectors

Workshop on the Risk Assessment of Transgenic Insects. A UNDP sponsored Workshop on the Risk Assessment of Transgenic Insects (Series 1) was co-hosted by Malaysia's Ministry of Natural Resources and Environment and the University of Malaya in November 2008.

The initiative was organised under the project Capacity Building for Implementation of Malaysia's Biosafety Act 2007, which has the objective to help consolidate Malaysia's national capacity for the implementation of the Cartagena Protocol on biosafety as well as the National Biosafety Act of 2007. The "Capacity Building" project in conjunction with the Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya organised a risk assessment workshop on transgenic insects to meet the goals in building capacity among regulators and scientists to undertake science based risk assessment in this new field. It is understood that this was the first workshop on transgenic insects in the world to prepare a case specific science based risk assessment, although individual countries have published risk assessments on transgenic insects, notably the USA (USDA, 2001; USDA, 2006; USDA, 2009). The workshop took place over three days and was attended by nearly 60 scientists and decision makers in Malaysia. The fields represented at the meeting included medical entomologists, vector control, virology, infectious diseases, medicine, and law along with national decision makers.

Participants discussed risk analysis, risk management and risk communication for three case studies: hypothetical open field release of genetically modified fruit flies, pink bollworm and mosquitoes. The introduced traits included a marker gene and repressible lethality for a population suppression strategy. The overall group was then divided into four working groups who independently prepared a risk assessment. In this role they were asked to play the role of a Non Governmental Organisation to determine the potential theoretical hazards associated with the hypothetical trials and then apply the tools of risk assessment and management as an Independent Biosafety Review Board to determine the likelihood and consequence of the identified potential hazards. The output of the risk assessment concluded that there were few potential hazards of such a hypothetical release and the hazards identified could be managed with appropriate risk management procedures (Beech *et al.,* 2009)

Future initiatives. At the time of writing the authors have learnt that additional initiatives for the risk assessment, management or communication on GM insects are in the early stages of preparation. The first of these is a Call to Tender by the European Food Safety Authority (EFSA) for Environmental Risk Assessment Criteria of Genetically Modified (GM) Insects to be placed on the EU market. (Supplement to the Official Journal of the European Union, 2009a and 2009b). The purpose of the tender is to select a contractor that can prepare a detailed analysis of the type of expertise and data required to conduct an environmental risk assessment of GM insects to be commercially released into the EU environment. The guidance is expected to cover the following categories of intended uses: (1) the control of animal and human disease-transmitting (vector) insects; (2) the control of agricultural pest insects; (3) the development of pathogen refractory GM insects; and (4) the production of products of interest. The contract is estimated to be granted mid to end of September 2009 and is expected to conclude around March 2010.

The UNDP and University of Malaya are planning a follow-up to their Risk Assessment Workshop series in the form of a number of public engagement meetings on biosafety and benefits of transgenic insects.

The MosqGuide project is planning a case study on national decision making regarding the use of GM vectors as an additional method for Aedes aegypti control, to reduce the numbers of mosquitoes that transmit dengue disease. Part of the case study includes a public consultation meeting, aimed at the technical community, which is anticipated to take place in Panama later in 2009.

CONCLUSION

We describe several initiatives based in various countries, regions or internationally, which consider biosafety, regulatory or ESC issues related to genetic strategies for insect control. While most are in the realm of guidance or guidelines, some have regulatory status and are legally binding. Each can provide useful background for upcoming national decisions regarding application of genetic strategies for vector control, as the most promising technologies move from laboratory to confined or open field trials and, if successful, eventual widespread field programmes for vectored disease control.

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REFERENCES

Ageep T.B., Cox J., Hassan M., Knols B.J.G., Benedict M. Q., Malcolm C. A., Babiker A., and El Sayed B.B. 2009. Spatial and temporal distribution of the malaria mosquito *Anopheles arabiensis* in northern Sudan: influence of environmental factors and implications for vector control. *Malaria Journal* 2009 8: 123 in press.

Alphey L., Beard C.B., Billingsley P., Coetzee M., Crisanti A., Curtis C., Eggleston P., Godfray C., Hemingway J., Jacobs-Lorena M., James A.A., Kafatos F.C., Mukwaya L.G., Paton M., Powell J.R., Schneider W., Scott T.W., Sina B., Sinden R., Sinkins S., Spielman A., Touré Y. and Collins F.H. 2002. Malaria control with genetically manipulated insect vectors. *Science* 298: 119–121.

Alphey L., Benedict M. Q., Bellini R., Clark G. G., Dame D., Service M., and Dobson S. 2009. Sterile-insect methods for control of mosquito-borne diseases – an analysis. *Vector Borne and Zoonotic Diseases* in press.

Alphey L. Natural and engineered mosquito immunity. *Journal of Biology* 2009, 8: 40.

Alphey L., Nimmo D., O'Connell S., Alphey N. Insect population suppression using engineered insects 2008. *In:* Transgenesis and the Management of Vector-Borne Disease (Ed. Aksoy, S) Landes Bioscience.

Angulo E., and Gilna B. 2008a. International law should govern release of GM mosquitoes. *Nature* 454: 158.

Angulo E., and Gilna B. 2008b. When biotech crosses borders. *Nature Biotechnology* 26: 277-282.

Beaty B. J., Prager D. J., James A. A., Jacobs-Lorena M., Miller L.H., Law J. H., Collins F. H. and Kafatos F. C. 2009. From Tucson to genomics and transgenics: The Vector Biology Network and the emergence of modern vector biology. *PLoS Neglected Tropical Diseases* 3(3).

Beech C. J., Nagaraju J., Vasan, S.S., Rose R.I., Othman R. Y., Pillai V., and Saraswathy, T.S. (on behalf of the Working Group) 2009. Risk analysis of a hypothetical open field release of a self-limiting transgenic *Aedes aegypti* mosquito strain to combat dengue. *Asia-Pacific Journal of Molecular Biology and Biotechnology* 17: 99-111.

Beeman R., Friesen K., and Denell R. 1992. Maternal-effect selfish genes in flour beetles. *Science* 256: 89.

Benedict M., D'Abbs P., Dobson S., Gottlieb M., Harrington L., Higgs S., James A., James S., Knols B., Lavery J., O'Neill S., Scott T., Takken and Toure Y. 2008 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* 8 2:127-167.

Benedict M. Q., and Robinson A. S. 2003. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends in Parasitology* 19: 349-355.

Brelsfoard C., Sechan Y., and Dobson, S. 2008. Interspecific hybridization yields strategy for South Pacific filariasis vector elimination. *PLoS Neglected Tropical Diseases* 2: e129.

Capurro M., Coleman J., Beerntsen B., Myles K., Olson K., Rocha E., Krettli A., and James A. 2000. Virus-expressed, recombinant single-chain antibody blocks sporozoite infection of salivary glands in Plasmodium gallinaceum-infected *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene* 62: 427-433.

Catteruccia F., Benton J., and Crisanti A. 2005. An Anopheles transgenic sexing strain for vector control. *Nature Biotechnology* 23: 1414-1417.

Chen C.-H., Huang H., Ward C., Su J., Schaeffer L., Guo M., and Hay B. 2007. A synthetic maternal-effect selfish genetic element drives population replacement in *Drosophila*. *Science* 316: 597-600.

Convention on Biological Diversity 1993<http://www.cbd.int/convention/convention.shtml>.

Convention on Biological Diversity 2009a Cartagena Biosafety Protocol; Status of Ratification and Entry into Force <http://www.cbd.int/biosafety/signinglist.shtml>.

Convention on Biological Diversity 2009b Report Of The First Meeting Of The Ad Hoc Technical Expert Group On Risk Assessment And Risk Management Under The Cartagena Protocol On Biosafety. <http://www.cbd.int/doc/meetings/bs/bsrarm-01/official/bsrarm-01-03-en.pdf>.

CNTBio 2005 Law 11.105 Safety norms and inspection mechanisms for activities that involve genetically modified organisms. <http://www.ctnbio.gov.br/index.php/content/view/12847.html>

Daegu Protocol 2007 New Biotechnologies for Pest and

Disease Control <http://biopesticide.ucr.edu/daegu/assets/DRAFT_Daegu_Protocol_12-11-06.pdf>.

Dyck V. A., Hendrichs J., and Robinson A. S., eds. 2005. Sterile Insect Technique: Principles and practice in Area-Wide Integrated Pest Management. *The Netherlands: Springer*. 787pp.

Federal Register 2006 United States Department of Agriculture, Animal and Plant Health Inspection Service [Docket No. APHIS-2006-0166] *In*: Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs: Draft Environmental Impact Statement (73 FR 3115).

Federal Register 2009 United States Department of Agriculture, Animal and Plant Health Inspection Service [Docket No. APHIS-2006-0166] *In*: Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs; Record of Decision (71 FR 21314-21316).

Franz, A., Sanchez-Vargas, I., Adelman, Z., Blair, C., Beaty, B., James, A., and Olson, K. 2006. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proceedings of National Academy of Sciences USA* 103: 4198-4203.

Heinrich J.C. and Scott M.J. 2000. A repressible female-specific lethal genetic system for making transgenic insect suitable for a sterile-release program. *Proceedings of the National Academy of Sciences of the USA* 97: 8229–8232.

IAEA 2006 Status and risk assessment of the use of transgenic arthropods in plant protection *Proceedings of a technical meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and the Secretariat of the International Plant Protection Convention*, Rome, 8 to 12 April 2002 IAEA TECDOC 1483.

James A. A. 2005. Gene driving systems in mosquitoes: rules of the road. *Trends in Parasitology* 21: 64–67.

Khongtak P., Vasan S. S., Ponlawat A., and Pilakasiri C. 2009. *In*: Mating competitiveness experiments in lab cages under total containment using imported RIDL OX-513A genetically sterile *Aedes aegypti* mosquitoes for research purpose. Bangkok: World Health Organization Collaborating Centre for Diagnostic Reference, Training and Investigation of Emerging Infectious Diseases.

Knipling E. 1955. Possibilities of insect control or eradication through use of sexually sterile males. *Journal of Economical Entomology* 48: 459-462.

Knols B.G.J., Louis, C. and Bogers, R.J. 2004. Bridging laboratory and field research for genetic control of disease vectors. 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>.

Lavery J., Harrington L., and Scott T. 2008. Ethical, social, and cultural considerations for site selection for research with genetically modified mosquitoes. *American Journal of Tropical Medicine and Hygiene* 79: 312–318.

Lee H.L., Vasan S.S., Nazni W.A., and Shahnaz M. 2008. Scientific report on the innovative application of *Aedes aegypti* RIDL-sterile insect technique to combat dengue and chikungunya in Malaysia. Kuala Lumpur: World Health Organization Collaborating Centre for Ecology, Taxonomy and Control of Vectors of Malaria, Filariasis and Dengue.

McMeniman C. J., Lane R. V., Cass B. N., Fong A. W. C., Sidhu M., Wang Y. F., and O'Neill S. L. 2009 Stable introduction of a life-shortening Wolbachia infection into the mosquito *Aedes aegypti*. *Science*. 323 5910: 141-144.

Mumford J., Quinlan M. M., Beech C. J., Alphey, L., Bayard, V., Capurro, M.L., Kittayapong, P., Knight, J.D., Marrelli, M.T., Ombongi, K., Ramsey, J.M., and Reuben, R. 2009. MosqGuide: A project to develop best practice guidance for the deployment of innovative genetic vector control strategies for malaria and dengue, *Asia-Pacific Journal of Molecular Biology and Biotechnology* 17: 93-95.

NAPPO 2006 NAPPO Newsletter: Importation and Release into the Environment of Transgenic Arthropods in NAPPO Member Countries p7-8. Available from: <http://www.nappo.org/Newsletter/Newsletters/NAPPOJune06-e.pdf>.

NAPPO 2007 *In*: NAPPO Regional Standards for Phytosanitary Measures RSPM 27: Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries <http://www.nappo.org/Standards/NEW/RSPM%20No.%2027-e.pdf>.

Pew Initiative on Food and Biotechnology, 2004. *In*: "Bugs in the System" Issues in the Science and Regulation of Genetically Modified Insects. A report from the Pew Initiative on Food and Biotechnology <http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Reports/Food_and_Biotechnology/pifb_bugs_012204.pdf>.

Phuc H. K., Andreasen M. H., Burton R. S., Vass C., Epton M. J., Pape G., Fu G., Condon K. C., Scaife S., Donnelly C. A., *et al*. 2007. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biology* 5: 11.

Riehle M.A., Srinivasan P., Moreira C.K., and Jacobs-Lore-

na, M. 2003. Towards genetic manipulation of wild mosquito populations to combat malaria: advances and challenges. *Journal of Experimental Biology* 206: 3809–3816.

Robinson A.S. 2002. Genetic sexing strains in medfly, *Ceratitis capitata*, sterile insect technique programmes. *Genetica* 116: 5–13.

Sinkins S., and Gould F. 2006. Gene drive systems for insect disease vectors. *Nature Reviews Genetics* 7: 427-435.

Singer P. A., Taylor A. D., Daar A. S., Upshur R. E. G., Singh J. A., Lavery J. V 2007 Grand Challenges in Global Health: The Ethical, Social and Cultural Program *PLoS Medicine* 4: e265.

Sperança M. A., and Capurro M. L. 2007. Perspectives in the control of infectious diseases by transgenic mosquitoes in the post-genomic era – A Review. *Memórias do Instituto Oswaldo Cruz* 10:, 425-433.

Supplement to the Official Journal of the European Union 2009a Tenders Electronic Daily. Parma; Defining the Environmental Risk Assessment criteria for Genetically modified (GM) insects to be placed on the EU market. <http://ted.europa.eu/Exec?DataFlow=N_one_doc_access.dfl&Template=TED/N_one_result_detail_curr.htm&docnumber=147726-2009&docId=147726-2009&StatLang=EN>.

Supplement to the Official Journal of the European Union 2009b Tenders Electronic Daily. Parma; Defining the Environmental Risk Assessment criteria for Genetically modified (GM) insects to be placed on the EU market Additional information. <http://ted.europa.eu/Exec?DataFlow=N_one_doc_access.dfl&Template=TED/N_one_result_detail_curr.htm&docnumber=155735-2009&docId=155735-2009&StatLang=EN>.

Scott T. W., Takken W., Knols B. G. J., and Boëte C. 2002. The ecology of genetically modified mosquitoes. *Science* 298: 117–119.

Takken W., Scott T. W., and Bogers R.J. 2002. Ecological aspects for application of genetically modified mosquitoes. *Proceedings of the Frontis Workshop on Ecological Challenges Concerning the Use of Genetically Modified Mosquitoes for Disease Control*, The Netherlands, 26–29 June 2002.

Thomas D. D., Donnelly C. A., Wood R. J., and Alphey L. S. 2000. Insect population control using a dominant, repressible, lethal genetic system. *Science* 287: 2474-2476.

Tindana P. O., Singh J. A., Tracy S., Upshur R. E. G., Daar A. S., Singer P. A., Frolic J., and Lavery J. V 2007 Grand Challenges in Global Health: Community Engagement in Research in Developing Countries. *PLoS Medicine* 4 9: e273.

USDA 2001 *In*: Availability of Environmental Assessment and Finding of No Significant Impact for Confined Field Test of Genetically Engineered Pink Bollworm Docket No. 01-024-2 <http://www.epa.gov/EPA-IMPACT/2002/January/Day-11/i741.htm>.

USDA 2006 *In*: Availability of an Addendum to an Environmental Assessment for Field Release of Genetically Engineered Pink Bollworm Docket No. APHIS–2006–0015 <http://www.aphis.usda.gov/brs/fedregister/BRS_20060620a.pdf>.

USDA 2008 *In*: Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs. Final Environmental Impact Statement – October 2008. <http://www.aphis.usda.gov/plant_health/ea/geneng.shtml>.

Vasan S. S. 2009. *In:* Deployment of innovative genetic vector control Strategies: Progress on regulatory and biosafety aspects and development of best-practice guidance. Proceedings of the 45th Annual Scientific Seminar (Grand Seasons Hotel, 18-19 March 2009). Kuala Lumpur: Malaysian Society of Parasitology and Tropical Medicine: 26-27.

WHO/TDR 1991. *In*: Report of the Meeting 'Prospects for Malaria Control by Genetic Manipulation of its Vectors, Tucson, USA, 27–31 January 1991, 68 pp. <http://stopdengue.hs.uci.edu/who.pdf>.

WHO/TDR 2009a *In:* Planning meetings on Progress and Prospects for the Use of Genetically Modified Mosquitoes to Prevent Disease Transmission: Meeting 1. Technical Consultations on the Current Status and Planning for Future Development. Geneva, May 4-6, 2009 <http://bch.cbd.int/database/record.shtml?id=48980%20>.

WHO/TDR 2009b *In:* Biosafety training course in India on genetically modified vector <http://apps.who.int/tdr/svc/grants/calls/biosafety-training-india>.

WHO/TDR 2009c *In*: Second African biosafety training course related to potential release of genetically modified disease vectors. <http://apps.who.int/tdr/svc/grants/calls/2nd-african-biosafety-training-course>.

Xi Z., Khoo C. C. H., Dobson S. L 2005. *Wolbachia* Establishment and Invasion in an *Aedes aegytpi* laboratory population. *Science* 310 5746: 326-328.