Chapter 9. The cartagena protocol and releases of transgenic mosquitoes

9.1 Introduction

The CBD came into force in 1993 with the aim of preserving the world's biological diversity.[1] Ever since the first LMOs/GMOs (for example, GM foods) were developed, transported and made commercially available, transgenic technology has provoked highly polarized views both within and between nations. Proponents have maintained that GM crops enhance nutrition and will balance future food shortages by increasing food supply; while opponents have cited risks to the environment and human health in addition to economic and legal concerns. [2] This rift is particularly apparent between the USA and the EU. The USA and countries that are heavily dependent on it for trade (e.g. several Latin American countries) are highly supportive of GM crops, whereas the EU and several of its former colonies (e.g. most African countries) are less supportive.[3]

Given these geopolitical differences in support and the potential for GMOs to cross international borders, Parties to the CBD called for a protocol to be developed to ensure "the safe transfer, handling and use" of GMOs. The protocol, which became known as "The Cartagena Protocol on Biosafety" was adopted on 29 January 2000 and entered into force on 23 September 2003.[4] As could be expected, UN Member States did not always agree on a number of issues, e.g. what should be covered by the Protocol, the extent to which the precautionary principle should be applied, and how the Protocol should relate to World Trade Organization (WTO) trade laws.[5] However, following negotiations, the Protocol was finalized and adopted in Montreal, Canada, on 29 January 2000, and opened for signature in Nairobi, Kenya, in 2000. It became legally binding after 90 days and once 50 countries had ratified it, i.e. on 11th September 2003.[4] As of November 2014, the Protocol has been ratified by 167 countries.[6] As an international treaty, it governs the biosafety concerns related to safe transboundary movement, transit, handling and use of GMOs/LMOs in order to protect biological diversity, human health and the environment from the risks posed by the deliberate release of LMOs into the environment.

The terms of the Protocol were primarily concerned with GM crops. However, at the same time, it was intended to apply to all GMOs (referred to as LMOs in the text). GMM technology is moving very quickly and, in recent years, open releases of LMMs have taken place in the Cayman Islands (2009–2010), Malaysia (2010), and Brazil (2011), with further releases being planned in other countries.[7,8] The release by Harris et al. in the Cayman Islands was the biggest (~3.3 million sterile male LMMs).[7] These releases have been of genetically sterile *Aedes aegypti*, the vector of dengue fever, and have demonstrated that large-scale releases of transgenic males can lead to reduced mosquito densities and, by implication, reduced dengue transmission.[7] Another landmark in this context is the recent release of *Wolbachia* to control mosquito-transmitted diseases such as dengue fever and chikungunya. *Wolbachia*, a maternally inherited intracellular bacterium, are of interest because, although not transgenic, the infected mosquitoes display important physiological changes which are inherited from one generation to the next, and are capable of spreading through populations and potentially across international borders.[9] This is a truly remarkable achievement by the scientists concerned.[10-12] The first open field trials of these mosquitoes took place in Queensland, Australia in 2011.[11] The open release was permitted in Australia, which

is not a signatory to the CPB, after the relevant national authorities performed risk assessments in line with the government's draft rules.[13] This chapter describes the application of the Protocol to GMMs and discusses the applicability of the Protocol to recent releases of sterile GMMs and mosquitoes infected with the *Wolbachia* bacterium.[14] Depending on the type of GMM technology being considered, the Protocol is either applicable or requires further consideration. In particular, there are a number of overarching issues regarding GMMs capable of spreading transgenes across national borders that are not covered by the text.[15] A sub-working group assigned by the CBD's AHTEG on Risk Assessment and Risk Management developed a document containing comprehensive risk assessment guidelines for GMMs,[16] but it does not address the overarching issues. In 2010, the CPB was strengthened with the adoption of a new international treaty called the "Nagoya–Kuala Lumpur Supplementary Protocol on Liability and Redress". Several countries are not signatories to the Protocol and, consequently, may not feel obliged to abide by it. There are, however, independent commentators, such as Marshall,[14] who feel that the Cartagena Protocol has weaknesses. They have suggested that it should be addressed prior to an open release of mosquitoes engineered with invasive gene drive systems**.** The chapter concludes with a discussion of the application of the Protocol to additional novel GMM technologies, and efforts that are currently underway to address the deficiencies of the Protocol in its present form.

9.2 Types of GMMs

Several strategies are being considered under two major types of effects, such as population suppression and population replacement, to control VBDs using GMMs which were explained in detail in Chapter 3. Some are well covered by the Protocol, and others require further elaboration.

9.2.1 Population suppression (sterile GMMs)

Strategies that target vector "demography/density" intend to reduce (suppress) the size of the mosquito population and thereby control pathogen transmission. These include methods to reduce the overall numbers of female mosquitoes, which will result in decreased reproduction. The strategy that has received the most interest to date is the release of GM sterile males that, upon mating with wild females, produce unviable offspring, thus resulting in population suppression.[17] This strategy benefits from the fact that male mosquitoes do not bite and so the large numbers of released GM sterile males decrease the disease-transmitting female population in the next generation without transmitting disease themselves. There are actually two variants of this technology – one in which both male and female offspring are rendered unviable by the sterility gene,[18] and another in which sterility is specific to female offspring.[19] The bi-sex lethal strategy has the benefit that transgenes are eliminated from the population within a generation or two; while the female-specific lethal strategy allows the sterility gene and, hence, the population suppressing effect, to persist for a few generations longer. The technology for this strategy has already been developed for *Aedes aegypti*,[18,19] and has been tested in open field trials in Brazil, the Cayman Islands and Malaysia. This technology is well covered by the Cartagena Protocol due to the fact that the transgenes are self-limited in time and space.

9.2.2 Population replacement (self-propagating GMMs)

Another strategy being developed involves the use of a "gene drive system" to spread disease-refractory genes into mosquito populations that target vector competence in order to reduce the inherent ability of individual mosquitoes to transmit a given pathogen.[20] This involves the introduction of engineered DNA and/or the manipulation of endogenous genes in order to inhibit pathogen replication within the mosquitoes, making them refractory to transmission of particular viruses or parasites. Upon release into the environment, these refractory GMMs will be expected to mate and introduce the change into the local mosquito population, "replacing" their ability to spread the targeted pathogen with a reduced or eliminated transmission capability. As a proof of principle for this strategy, a malaria-refractory gene has been engineered in *Anopheles stephensi*, the vector of rodent malaria, which works by preventing the passage of the malaria parasite through the mosquito midgut following ingestion and through the mosquito salivary glands.[21] A dengue-refractory gene has also been engineered in *Ae. aegypti* that takes advantage of the natural antiviral pathway in the mosquito, placing it under the control of a blood-meal specific promoter.[22] Other approaches are also being explored, such as the expression of antibodies that kill malaria parasites within the mosquito,[23] and the discovery of genes that govern disease-refractoriness in natural mosquito populations.[24]

Progress has also been made in developing gene drive systems to spread these genes into mosquito populations. One of the early inspirations for this strategy was the observation that a transposable element known as the *P* element was observed to spread through the worldwide population of *Drosophila melanogaster* in just a few decades simply through biasing inheritance in its favour.[25] This led to the idea that a disease-refractory gene could "hitchhike" such a system. Since then, a synthetic gene drive system known as *Medea* has been engineered in *D. melanogaster*.[26] Progress has been slow at engineering the *Medea* system in *Ae. aegypti*. However, another gene drive system known as a HEG has been engineered in *Anopheles gambiae*, the primary malaria vector in sub-Saharan Africa.[27]

If gene drive systems such as these can be stably linked to disease-refractory genes, then just a few GMMs with these constructs would be capable of propagating transgenes over the entire geographical range of the species. This has far-reaching implications for wide-scale disease control. However, the application of the Cartagena Protocol to this technology is more problematic because the gene drive systems would be capable of spreading transgenes across international borders regardless of whether neighbouring countries were supportive of the technology.

9.3 Application of the Cartagena Protocol to GMMs

9.3.1 Definition of the terms of the Cartagena Protocol

Article 4 of the Cartagena Protocol states that the Protocol applies to, "the transboundary movement, transit, handling and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health." There are a few terms in this definition that require definition themselves. An LMO is essentially a GMO that is living and is defined in the Protocol as "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology" (Article 3). A living organism is defined as "any biological entity capable of transferring or replicating genetic material" (Article 3) and, of particular interest, is the definition of "modern biotechnology," which encompasses "the application of in vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acids into cells or organelles… that overcome

natural physiological reproductive or recombination barriers" (Article 3). Finally, "transboundary movements" include movements of LMOs both between Parties of the Protocol and between Parties and non-Parties (Article 3). Taken together, this means that the Protocol applies to the international movement of GMMs provided that at least one of the two countries is a Party to the Protocol.

9.3.2 Protection against an accidental release

The Cartagena Protocol was, in part, written with the intent of protecting developing countries against threats to biosafety due to a lack of resources to conduct their own risk assessment. The Advance Informed Agreement (AIA) procedure – the centrepiece of the Protocol – allows an importing country to demand the exporting country to both conduct a risk assessment and bear the costs of this assessment. During negotiations, however, it was decided that GMOs in transit or destined for contained use were exempt from this procedure (Article 6). Countries with strong biotech industries argued that GMOs in transit and containment pose negligible risks and hence the AIA procedure restricts trade unnecessarily.[5]

This exemption may be acceptable for GM crops and sterile varieties of GMMs; however it begs re-examination for GMMs with invasive gene drive systems. It is likely that such mosquitoes will undergo phased testing, beginning with laboratory studies of their basic characteristics and testing for adverse effects.[28] Following this, contained field cage trials are likely as an intermediate between laboratory studies and an open release. The fact that the AIA procedure does not apply to LMOs destined for contained use means that it may not apply to GMMs destined for field cage trials and, hence, the importing country (most likely a disease-endemic country – DEC - and possibly a developing one) is not entitled to require the exporting country to conduct a risk assessment at its own expense. The significance of this exemption is elevated for GMMs with invasive gene drive systems due to the fact that flying insects are particularly difficult to contain, and breaches of containment are impossible to rule out. In addition to this, once released, GMMs with gene drive systems could spread transgenes on a large scale prior to assessment of risks in that environment. Given the inability of some countries to conduct a sufficiently detailed risk assessment on their own, it has been argued that the Protocol should be adapted so that GMMs in transit or destined for contained use are covered by the AIA procedure.[15]

9.3.3 Movement of GMMs between non-Parties

Another situation where the protections of the Protocol do not apply is for movements of GMMs between non-Parties to the Protocol. Of particular relevance, the USA and several other countries with large biotech industries are not signatories, such as Australia, Argentina and Canada. Additionally, several developing countries with endemic mosquito-borne diseases are yet to sign the Protocol. These include in Africa: Cote d'Ivoire, Equatorial Guinea, Sao Tome and Principe, and Sierra Leone; and in South America: Chile and Uruguay.[6] The concern here is that, for GMMs originating in countries such as Australia and the USA, several DECs are not protected by the Protocol at all. Countries with strong biotech industries have refused to sign the Protocol because they claim it allows environmental precaution to provide an excuse for violation of WTO trade laws.[5] This may be relevant to GM crops, but is completely irrelevant for self-propagating GMMs, highlighting the problem of having a single Protocol that applies collectively to all GMOs.

The severity of these concerns is heightened by the fact that the emergency response and liability measures listed in the Protocol are inadequate for dealing with GMMs with gene drive systems. According to the Protocol, if a GMM travels from one country to another, then the recipient country may ask the country where the release occurred to "dispose, at its own expense," the GMMs "by repatriation or destruction, as appropriate" (Article 27). All Parties to the Protocol are

also required to prevent GMMs from crossing into their borders illegally. These measures are impractical for mosquitoes and many other disease vectors with gene drive systems.

9.3.4 Provision for an intentional release

GMMs with gene drive systems present a quandary for the Cartagena Protocol due to their ability to propagate transgenes across international borders in the absence of an international agreement. As discussed earlier, the Protocol seems to offer inadequate protection against an accidental release of GMMs with gene drive systems; however, its requirements for an intentional release seem very difficult to satisfy. The AIA procedure applies prior to the first environmental release of GMMs in another country (Article 7) and, under this procedure, the importing country may request the exporting country to perform a risk assessment at their own expense (Article 15), part of which is to determine the likelihood of unintentional transboundary movement (Article 16). Furthermore, the Protocol states, "Each Party shall take appropriate measures to prevent unintentional transboundary movements of LMOs" (Article 16). For GMMs with gene drive systems, unintentional movements are very difficult to prevent, which questions the practically of approving an environmental release.

While this is an important restriction for a technology that has not been tested, it would be disappointing to rule out a technology with the potential to control diseases on a global scale. One solution would be a multilateral agreement regarding GMMs with invasive gene drive systems. The Cartagena Protocol has a provision for bilateral, regional and multilateral agreements that are "consistent with the objective of this Protocol" and "do not result in a lower level of protection than that provided for by the Protocol (Article 14)." An agreement on GMMs with gene drive would have to acknowledge that any environmental release is intentionally international.

The problem with a multilateral agreement would be its scale and feasibility. Gene drive systems have the potential to spread through a species wherever they exist in the world. Consequently, an agreement on GMMs with invasive gene drive systems would potentially require the compliance of every country that the vector species inhabits. The possibility of achieving this is questionable given that, in 2002, Zambia rejected GM food aid from the USA during a famine that threatened hundreds of thousands of lives simply because it was genetically modified.[29] However, it is possible that GMMs may be more widely acceptable than GM crops given that their purpose is to improve health without an overt profit motive. In support of this hypothesis, medical applications of genetic engineering (such as insulin-producing GM bacteria) have much higher acceptability ratings than GM crops.[30]

9.4 Application to *Wolbachia*-infected mosquitoes

With the regulatory difficulties associated with releasing GMMs with invasive gene drive systems, one alternative is to escape the definition of "modern biotechnology" and hence the application of the Protocol altogether. The text of the Cartagena Protocol states that it applies strictly to living organisms engineered through the use of in vitro nucleic acid techniques (Article 3). Organisms with gene deletions produced by traditional means are, therefore, exempt as are mosquitoes infected with a non-transgenic strain of the *Wolbachia* bacterium. Some commentators (e.g. Macer[31]) have drawn attention to Article 5 of the Protocol which states that GM organisms considered "pharmaceuticals for humans" are exempt provided they "are addressed by other relevant international agreements or organizations", although the interpretation of GMMs as pharmaceuticals is not widespread.

CHAPTER 9
The cartagena protocol and releases of transgenic mosquitoes

Ideally, modified mosquitoes should be regulated on the basis of the physiology of their modification rather than the process by which the modification is achieved. However, since the Cartagena Protocol applies specifically to GMOs, then mosquitoes infected with a non-GM strain of *Wolbachia* are exempt from its application.[15] *Ae. aegypti* mosquitoes have recently been stably infected with a natural mutant strain of *Wolbachia* known as *wMelPop*,[32] which provides an interesting case study because, although no genetic modification is involved, the modification leads to a range of physiological effects such as reductions in mosquito lifetime, dengue viral load and biting ability with age.[9] The stability of the *Wolbachia* infection essentially means that an entire bacterial genome has been assimilated into the mosquito. Additionally, its manner of inheritance allows it to propagate into a mosquito population and potentially spread across international borders much like a gene drive system.[9] However, despite all of these factors, there are no international regulations that apply to non-GM *Wolbachia*-infected mosquitoes because they do not satisfy the Cartagena Protocol's definition of "modern biotechnology". It would be unfortunate if a method of modification were chosen first and foremost for its immunity to excessive regulatory requirements, rather than on the basis of its safety and efficacy.

9.5 Implications of recent releases of sterile GM and *Wolbachia*-infected mosquitoes

Research into GM and *Wolbachia*-infected mosquitoes is progressing extremely quickly, with releases of sterile GMMs occurring since 2009, and releases of *Wolbachia*-infected mosquitoes occurring since 2011. The first sterile GMM releases were of GM *Ae. aegypti* mosquitoes carrying RIDL in the Cayman Islands.[7] Since then, releases have occurred in Brazil and Malaysia.[33,34] These allow us to assess the suitability of the Protocol to self-limiting strains of GMMs.

The first *Wolbachia*-infected releases were of *Ae. aegypti* mosquitoes infected with the *wMelPop* strain of *Wolbachia* (henceforth referred to as *wAe. aegypti*).[11] This is a non-transgenic strain of *Wolbachia*,[32] and, hence, does not fall within the remit of the Cartagena Protocol. However, it does have several properties in common with GMMs with gene drive systems and, thus, highlights questions of relevance to GMMs with gene drive systems.

9.6 Releases of sterile GMMs

The strain of GMM released in the Cayman Islands field trial (OX513A) consisted of a repressible sterile phenotype that affects both male and female offspring of transgenic individuals.[18] Large-scale releases of males of this strain are expected to reduce dengue transmission because wild females that mate with transgenic males produce no viable offspring, thus reducing the population size of the disease vector. The Mosquito Research and Control Unit (MRCU) implemented the first Cayman Islands releases with the Oxitec Ltd. strain OX513A in November–December 2009 to assess the competitiveness of transgenic males in the field. This was followed by a large-scale release of transgenic males in May–October 2010 to test for population suppression.[35] Results from these trials suggest an up to 80% reduction in the local Ae. aegypti population ~11 weeks following commencement of the trial which was sustained until the trial ended.[7]

More recently, the Institute for Medical Research (IMR) in Malaysia carried out another open field trial in December 2010–January 2011 using the same OX513A strain, and a large-scale release of the same strain has been taking place in Brazil since February 2011.[34] The Malaysian release took place in an uninhabited area of Bentong to assess the dispersal

and longevity of transgenic males in the field.[33] The Brazilian release took place in the city of Juazeiro, resulting in the release of more than 10 million mosquitoes over the course of the last year.[8] Another self-limiting strain engineered with a female-specific flightless phenotype[19] has undergone successful contained trials and is being evaluated.

As Benedict and Robinson[36] have argued, it is appropriate that the first releases of transgenic mosquitoes were of genetically sterile males. Male mosquitoes do not bite, reducing the risks to human health; and sterile males produce unviable offspring, thus reducing the population of disease-transmitting female mosquitoes in subsequent generations. Furthermore, because the released transgene encodes sterility, it is only expected to persist in the wild for a few generations following a release. This minimizes biosafety issues because, provided that the GMMs are not released at the border between two countries, they will remain confined to the country of release. The Cartagena Protocol then applies to the import of GMMs intended for release into the environment, and requires that Parties make decisions on this import based on a scientifically sound risk assessment (Article 15).

In terms of the sterile GMMs developed by Oxitec Ltd., a risk assessment was conducted for the strain released into the Cayman Islands and Malaysia following a UNDP-sponsored Workshop on the Risk Assessment of Transgenic Insects in Malaysia in November 2008.[37] This Workshop assessed 31 risks of a hypothetical open field release of the bi-sex lethal GMM strain (OX513A), and has been expanded upon by Patil et al.[38] who identified an additional two risks for this strain, and another eight risks for a strain engineered with a repressible female-specific flightless phenotype.[19] All of the potential risks were determined to be of either low or negligible magnitude. One of the most serious concerns was that suppression of *Ae. aegypti* populations could lead to their replacement with *Aedes albopictus* and a consequent increase in chikungunya transmission. However, this risk was considered to be of low magnitude because releases of sterile *Ae. aegypti* are not expected to result in population extinction, and the long-term strategy would be to suppress both *Ae. aegypti* and *Ae. albopictus* populations.[37] This risk assessment satisfies the requirement, mandated by the AIA procedure (Articles 8–10 and 12) that the exporting country perform a risk assessment at its own expense, if requested by the importing country, prior to the first transboundary movement of a GMO intended for release into the environment.

One weakness of the Cartagena Protocol, highlighted by exports of GMM eggs by Oxitec Ltd. to the Cayman Islands (a British Overseas Territory), Malaysia and several other destinations, is that the AIA procedure does not apply to GMOs destined for contained use (Article 7). In almost all cases, GMMs are first exported for careful analysis in laboratory studies and cage trials in the receiving country, and the importing country is not entitled to request the exporting country to perform a risk assessment under these circumstances. Therefore, although risk assessments were performed for the GMM strains developed by Oxitec Ltd.,[37,38] it is not clear that these were required for exports to Brazil, France, India, Malaysia, Singapore, the USA and Viet Nam. Initial containment effectively sidesteps the risk assessment requirement because, once the GMMs have been received by the importing country, the country is free to release them into the environment in accordance with their own national regulations.

Releases of Oxitec Ltd. GMMs by the MRCU in the Cayman Islands also highlight confusion over the applicability of the Cartagena Protocol to transboundary movements between Parties to the Protocol and their overseas territories. As an overseas territory of the United Kingdom, the Cayman Islands are not able to ratify UN protocols independently of the United Kingdom. Parliamentary discussions suggest that overseas territories are not considered to be the same Party as the United Kingdom, but they are encouraged to become a Party to the United Kingdom's instrument of ratification of the Protocol.[39] The Cayman Islands have not done this and are, therefore, considered to be a non-Party. Parliamentary discussions further suggest that the provisions of the Protocol do not apply to transboundary movements of LMMs

from the United Kingdom (a Party) to the Cayman Islands (a non-Party).[39] However, this contradicts Article 24 of the Protocol, which states, "transboundary movements of LMOs between Parties and non-Parties shall be consistent with the objective of this Protocol."

Regardless of the applicability of the Cartagena Protocol, the export of GMMs to the Cayman Islands was subject to a similar European Commission regulation (EC Regulation 1946/2003), which requires that the exporter notify the competent authority in the importing country of the first transboundary movement of a GMO, and to await its consent to proceed.[39] The decision to release the GMMs into the environment is then subject to local legislation. Parties to the Protocol are required to notify the Biosafety Clearing House (BCH) of decisions to import or release GMOs into the environment (Article 20), and Malaysia has done this. However, since the Cayman Islands are a non-Party to the Protocol, they are not required to make such a notification.

The release of GMMs into the environment in the Cayman Islands and Malaysia was greeted with some controversy.[33,40–43] The major criticisms concerned the manner in which information about the trials was disseminated – an issue that is beyond the scope of the Cartagena Protocol. In both cases, the degree of community engagement was questioned, and several groups complained that they had not been given advanced information about the releases. Additionally, for the Cayman Islands, a video was posted on YouTube describing the trial, but it did not mention that the released mosquitoes were GM. Nevertheless, the releases did abide by national regulations in both cases. In the Cayman Islands, the trial abided by a draft biosafety bill that had yet to become law, the MRCU obtained a permit from the Cayman Islands Department of Agriculture, and a risk analysis and environmental impact assessment were carried out.[39] In terms of community engagement, elected political representatives were educated, information about the trial was sent to local newspapers, and flyers were distributed among the local population.[41]

In Malaysia, Oxitec Ltd. and the IMR worked closely with the Malaysian Government in assessing risk factors examined by the Genetic Modifications Advisory Committee.[44] The Natural Resources and Environment Ministry placed advertisements about the trial in local newspapers on 5 and 9 August 2010[45] and nine NGOs were invited to provide feedback during a one-month public feedback period from 5 August to 4 September 2010.[46] The terms of the trial were publicized on the IMR's website for a month, and the IMR put up notices in the trial area three weeks in advance.[45] Permission was obtained from local authorities, and the IMR held two public talks with local people – one in collaboration with the Bentong Municipal Council and another with the Bentong Malaysian Chinese Association.[46] Community engagement requirements for the first release were reduced because the trial was carried out in an uninhabited area. Despite this, negative reactions were encountered, particularly from NGOs and the media. Lessons should be learned from their criticisms, while acknowledging that the first use of a transgenic technology may always be greeted with some opposition.

9.7 Releases of *Wolbachia*-infected mosquitoes

Releases of *Wolbachia*-infected mosquitoes in Queensland, Australia, in January 2011 highlighted questions of relevance to self-propagating varieties of GMMs.[47,48] These were the first open releases of *wAe. aegypti* and were greeted with much less controversy than releases of genetically sterile mosquitoes, probably because the *Wolbachia* infection did not involve genetic modification. This also meant that there were fewer regulatory hurdles to overcome prior to releasing the *wAe. aegypti* mosquitoes into the environment,[49] making them an attractive alternative to GMMs with gene drive systems. Since the Cartagena Protocol does not apply to mosquitoes infected with non-transgenic strains of *Wolbachia*,

the Australian release was subject to national regulations, but was not subject to international regulations regarding subsequent potential transboundary movements.

When the Eliminate Dengue Program at the University of Queensland sought approval to release *wAe. aegypti* into the environment, the situation was described by regulators as a "regulatory no man's land".[50] Both *Wolbachia* and *Ae. aegypti* were already present in Australia, so the release did not constitute the introduction of a new species. Furthermore, the Office of the Gene Technology Regulator ruled that *wAe. aegypti* was not a GMO, and so could not be regulated under the Gene Technology Act. In the end, a submission was presented to the Primary Industries Ministerial Council, following which it was concluded that the Australian Pesticides and Veterinary Medicines Authority (APVMA) provided the most appropriate regulatory framework: *wAe. aegypti* was considered to be a "veterinary chemical product" on the basis that *Wolbachia* is a "substance that is used for application to an animal… as a way of directly or indirectly modifying the physiology of the animal so as to alter its natural development or reproductive capacity."[51] A decision was taken to approve the trial based on the results of a prior risk assessment undertaken by the Commonwealth Scientific and Industrial Research Organisation (CSIRO)[52] and a risk assessment focusing on environmental impact undertaken by the APVMA with support from the Federal Commonwealth Government's Department of Environment, Water, Heritage and the Arts. The trial was monitored by the APVMA.

As pointed out by De Barro et al.[50], the Australian regulatory framework is rigorous. Nevertheless, a major weakness is that, if the trial is successful, *wAe. aegypti* is predicted to spread throughout Australia and possibly beyond, while only Australia was considered in the regulatory approval. The same weakness is reflected in the risk assessment conducted by the CSIRO.[52] Here, a total of 50 risks were identified following community engagement exercises, a workshop and email solicitation with a dengue consultation group. The CSIRO acknowledged that, if successful, "*Wolbachia Ae. aegypti* will be self-sustaining after the inoculative release and… will be driven into the Australia *Ae. aegypti* populations…".[52] However, the possibility that the strain may spread into other countries was not considered.[53] It may be argued that, since the release is judged safe by Australia's rigorous standards, it will also be safe for other countries. However, this assumes that the standards of one country apply to another, which is not necessarily the case. Nevertheless, there are no international regulations that apply to non-transgenic *Wolbachia*-infected mosquitoes, and so, even if a release is expected to have international implications, the decision to release is a national one.

Furthermore, even if *wAe. aegypti* was considered to be a GMO and within the remit of the Cartagena Protocol, the significance of the Protocol would be undermined by the fact that Australia is not a signatory. Like many countries with strong biotech industries, such as Argentina and the USA, Australia is reluctant to sign the Protocol because it may restrict trade, partly because a strong interpretation of the precautionary principle may allow economic protectionism to masquerade as environmental protection.[5] Article 24 states that the Protocol also applies to "transboundary movements of LMOs between Parties and non-Parties", and a release of *wAe.aegypti* in Australia could conceivably spread into any number of Parties to the Protocol (for instance, Papua New Guinea). However, it seems overly optimistic that a non-Party would feel obliged to abide by a Protocol to which it did not agree. The Nagoya-Kuala Lumpar Supplementary Protocol on Liability and Redress[54] applies to "damage resulting from transboundary movements of LMOs", and includes movements originating from non-Parties in its scope (Article 3 of the Supplementary Protocol). Yet, it is unclear whether the liability procedures (Article 12 of the Supplementary Protocol) would provide an adequate incentive to prevent a non-Party from releasing a self-propagating LMO on their own accord. In essence, even if the Protocol did apply to *wAe. aegypti*, the Australian release may still have occurred.

CHAPTER 9
The cartagena protocol and releases of transgenic mosquitoes

9.8 Application of the Protocol to novel self-propagating GMM technologies

The regulatory approaches that governed the releases of genetically sterile and *Wolbachia*-infected mosquitoes have significant implications for novel varieties of self-propagating GMMs, which are also capable of propagating transgenes across national borders. Of particular concern, the AIA procedure of the Cartagena Protocol is not expected to apply to many imports of self-propagating GMMs because the first mosquitoes will likely undergo cage trials in the receiving country to assess their behaviour in the ambient environment in a location that the species naturally inhabits.[28] Under these circumstances, the importing country will not be entitled to request the exporting country to perform a risk assessment at their own expense, which is a concern for developing countries with a lack of resources to conduct a comprehensive risk assessment of their own.[15] As for the sterile GMMs developed by Oxitec Ltd., it is likely that a risk assessment for self-propagating GMMs will be conducted anyway, either voluntarily or as required by national or regional regulations in the exporting country. However, it is of concern that the Cartagena Protocol does not mandate a risk assessment under the most likely scenario in which a release follows contained study.

Another concern is that, although a release of GMMs with invasive gene drive systems could propagate transgenes over entire continents, the Cartagena Protocol may not be strong enough to prevent some countries from acting unilaterally in a decision to release them. A strict interpretation of the Protocol suggests that a release of GMMs with invasive gene drive systems would require a multilateral agreement between all affected nations – a difficult task considering the potential scale of spread.[15] However, the unilateral decision by a non-Party to the Protocol, in this case Australia, to release *wAe. aegypti* mosquitoes, which are expected to propagate across national borders, highlights the possibility that GMMs with invasive gene drive systems could also be released in the absence of an international agreement. Such a release could occur because: (i) a non-Party may not feel obliged to abide by the terms of a protocol to which it did not agree; (ii) the likelihood of damage to a Party resulting from an unintentional transboundary movement may be considered minimal; and (iii) the liability measures outlined in the Supplementary Protocol may be inadequate to dissuade a non-Party (or even a Party) from conducting a release on their own accord. Several DECs, such as Cote d'Ivoire, Equatorial Guinea, Sao Tome and Principe, and Sierra Leone, have yet to sign the Protocol, in addition to the countries with strong biotech industries, mentioned earlier.

9.9 *Medea* and X-shredders

Issues relating to self-propagating GMMs should be taken seriously, as work is currently ongoing to create *Medea* elements capable of driving disease-refractory genes (e.g. Ito et al.; Franz et al.[21,22]) into mosquito populations in order to render entire vector populations refractory to disease.[26] Work is also ongoing towards the development of an X-shredder which utilizes a HEG to cleave the X chromosome during spermatogenesis,[55] thus creating a bias towards Y-bearing spermatozoa and eventually leading to an all-male population crash.[56] Both systems are capable of spreading across national borders, with the latter strategy inducing a cascade of population crashes in its wake. Interestingly, the goal of creating an X-shredder in *An. gambiae* has led to the intermediate creation of a GM sterile male, which could be used for self-limiting population suppression field trials in sub-Saharan Africa.[55]

9.10 Gene drive systems with thresholds

Finally, work is ongoing towards the creation of gene drive systems that will only spread into a population if they exceed a critical population frequency.[57–59] These systems have three desirable features for biosafety when the goal is local population replacement – accidentally released mosquitoes are unlikely to persist in the wild because they will inevitably be present at sub-threshold levels; mosquitoes released at super-threshold frequencies at an isolated release site are expected to spread transgenes locally while remaining at sub-threshold levels at nearby locations; and transgenes can be eliminated from the release site through a sustained release of wild mosquitoes diluting transgenes to sub-threshold levels. These desirable features are acknowledged in the first guidance document of the Sub-Working Group on LMMs,[16] although a proper ecological assessment will be required on a case-by-case basis when such a release is considered.

9.11 Discussion

Ostera and Gostin[60] have argued for a new international treaty governing the environmental release of genetically or biologically modified disease vectors. A dedicated treaty would certainly be able to address the unique biosafety concerns posed by GM and *Wolbachia*-infected mosquitoes, but any treaty will inevitably be faced with the problem that there will be non-signatories who may choose to release self-propagating modified mosquitoes on their own accord. Given that releases of GMMs have already begun, an adaptation of the Cartagena Protocol that quickly addresses the pressing needs of GMMs provides a better solution, particularly considering that the Protocol already has 167 signatories – a total which has taken more than a decade to achieve.

The exemption from the AIA procedure of GMMs being considered for release following initial laboratory studies and/or cage trials is a major weakness of the Protocol. This issue is important to address prior to the development of GMMs with invasive gene drive systems because their risks are magnified by the ability of transgenes to spread globally; however a risk assessment is not currently mandated under the most likely release scenario. Another weakness is that it may currently be impossible to release GMMs with invasive gene drive systems in a manner that is consistent with the Protocol if one of the countries into which they may spread is fundamentally opposed to GMOs.[15] A mechanism for the independent review of GMOs with international implications for biodiversity and/or human health should be considered in these cases,[60] since a paralytic Protocol is less likely to be upheld. Such a review should consider both the risks and benefits of LMMs,[61] and the promise that GMMs have for reducing the global burden of vector-borne diseases.

It will be difficult to encourage non-Parties to abide by the terms of the Protocol no matter what changes are made. Nevertheless, clarification should be provided to Parties of the Protocol that the Protocol does apply to exports of GMOs to non-Parties. Clarification should also be provided that the Protocol does apply to mosquitoes infected with transgenic strains of *Wolbachia*, if such strains prove useful.

9.12 Conclusion on efforts to address the deficiencies of the Protocol

Further discussions will be required to guide the evolution of the Cartagena Protocol, and lessons learned from recent releases will ensure that it moves in the right direction to address the unique biosafety concerns posed by self-limiting and self-propagating LMMs. For the time being, modifications to the Protocol have taken the form of an additional guidance document that serves as an extension of Annex III of the Protocol on risk assessment, with a specific section on GMMs. The guidance document was written by an AHTEG-assigned Sub-Working Group on LM Mosquitoes. The first draft of the document was presented to the Fifth Meeting of the Conference of the Parties Serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety, which took place in Nagoya, Japan, in October 2010.[16]

This guidance document is an important first step towards incorporating the biosafety issues posed by GMMs into the Cartagena Protocol. It raises a number of important considerations regarding risk assessment that may be largely adequate for releases of sterile and self-limiting GMMs. However, for strategies involving mosquitoes capable of replacing entire populations with disease-incompetent varieties, several issues still need to be resolved. For these strategies, a balance must be sought between the precautionary principle, respect for the sovereignty of states, and the ethical mandate to prevent disease on a global scale. Further discussion is needed to address the international regulatory challenges posed by GMMs in working towards the goal of global VBD control.

REFERENCES

1. Convention on Biological Diversity. New York, NY: United Nations; 1992 (http://www.cbd.int/doc/legal/cbd-en.pdf, accessed 7 November 2014).
2. Specter M. The pharmageddon riddle: did Monsanto just want more profits, or did it want to save the world? The New Yorker. 10 April 2000 (http://www.michaelspecter.com/2000/04/the-pharmageddon-riddle/, accessed 6 November 2014).
3. James C. Global status of commercialized biotech/GM crops: 2012. Ithaca, NY: Institute for Service for the Acquisition of Agri-biotech Applications; 2012.
4. Secretariat of the Convention on Biological Diversity. Cartagena Protocol on Biosafety to the Convention on Biological Diversity. 2000 (http://bch.cbd.int/protocol/text/, accessed 6 November 2014).
5. Cosbey A, Burgiel S. The Cartagena Protocol on Biosafety: an analysis of results. Winnipeg: International Institute for Sustainable Development; 2000.
6. Convention on Biological Diversity. List of Parties. (http://www.cbd.int/information/parties.shtml#tab=1, accessed 6 November 2014).
7. Harris A, Nimmo D, McKemey A, Kelly N, Scaife S, Donnelly CA et al. Field performance of engineered male mosquitoes. *Nature Biotechnol.* 2011;29:1034–7.
8. Mendes H. Brazil tests GM mosquitoes to fight dengue. Sci Dev Net. 10 April 2012 (http://www.scidev.net/en/health/genomics/news/brazil-tests-gm-mosquitoes-to-fight-dengue.html, accessed 6 November 2014).
9. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and Plasmodium. *Cell* 2009;139: 1268-1278.

10. McMeniman CJ, O'Neil SL. 2010. A virulent *Wolbachia* infection decreases the viability of the dengue vector *Aedes aegypti* during period of embryonic quiescence. *PLoS Negl Trop Dis.* 2010;4:e748.

11. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 2011;476:454–7.

12. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ et al. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 2011;476:450–3.

13. Gilbert N. Letting the bugs out of the bag. *Nature* 2011;470:139.

14. Marshall JM. The Cartagena Protocol in the context of recent releases of transgenic and *Wolbachia*-infected mosquitoes. *AsPac J Mol Biol Biotechnol.* 2011;19:93–100.

15. Marshall JM. The Cartagena Protocol and genetically modified mosquitoes. *Nat Biotech*. 2010;28:896–7.

16. Final Report of the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety. Ad Hoc Technical Expert Group on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety. Ljubljana, 20–23 April 2010. (http://www.cbd.int/doc/meetings/bs/bsrarm-02/official/bsrarm-02-05-en.pdf, accessed 6 November 2014).

17. Alphey L. Re-engineering the sterile insect technique. *Insect Biochem Mol Biol.* 2002;32:1243–7.

18. Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G et al. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol.* 2007;5:11.

19. Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P. Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci USA*. 2010;107:4550–4.

20. Marshall JM, Taylor CE. Malaria control with transgenic mosquitoes. *PLoS Med.* 2009;6:e1000020.

21. Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* 2002;417:452–5.

22. Franz AW, Sanchez-Vargas I, Adelman N, Blair CD, Beaty BJ, James AA et al. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proc Natl Acad Sci USA*. 2006;103:4198–203.

23. De Lara Capurro M, Coleman J, Beerntsen BT, Myles KM, Olson KE, Rocha E et al. Virus-expressed, recombinant single-chain antibody blocks sporozoite infection of salivary glands in Plasmodium gallinaceum-infected *Aedes aegypti*. *Am J Trop Med Hyg.* 2000;62:427–33.

24. Riehle MM, Markianos K, Niare O, Xu J, Li J, Touré AM et al. Natural malaria infection in *Anopheles* gambiae is regulated by a single genomic control region. *Science* 2006;312:577–9.

25. Engels WR. The P family of transposable elements in Drosophila. *Ann Rev Genet.* 1983;17:315–44.

26. Chen CH, Huang H, Ward CM, Su JT, Schaeffer LV, Guo M et al. A synthetic maternal-effect selfish genetic element drives population replacement in Drosophila. *Science* 2007;316:597–600.

27. Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H, Ulge UY et al. 2011. A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. *Nature* 2011;473:212–15.

CHAPTER 9
The cartagena protocol and releases of transgenic mosquitoes

28. Benedict MQ, D'Abbs P, Dobson S, Gottlieb M, Harrington L, Higgs S 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 Zoonotic Dis.* 2008;8:127–66.

29. Bohannon J. Zambia rejects GM corn on scientists' advice. *Science* 2002:298:1153–4.

30. Masakazu I, Macer D. Attitudes to biotechnology in Japan in 2003. *EJAIB.* 2003;13:78–90.

31. Macer D. Ethical, legal and social issues of genetically modified disease vectors in public health. Geneva: World Health Organization; 2003.

32. McMeniman CJ, Lane RV, Cass BN, Fong AWC, Sidhu M, Wang YF et al. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* 2009;323:141–4.

33. Malaysia releases GM mosquitoes in landmark trial. *The Independent*, 27 January 2011.

34. Cristino LG. Bahia inicia uso de inseto transgenico contra dengue [Bahia initiates use of transgenic insect against dengue]. 24 February 2011. Folha.com (http://www1.folha.uol.com.br/ciencia/880408-bahia-inicia-uso-de-inseto-transgenico-contra-dengue.shtml, accessed 7 November 2014).

35. Wilson S. MRCU looks to modify mozzies. Cayman Compass.com, 2 October 2009 (http://www.compasscayman.com/caycompass/2009/10/01/MRCU-looks-to-modify-mozzies/, accessed 6 November 2014).

36. Benedict MQ, Robinson AS. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends Parasitol.* 2003;19:349–55.

37. Beech CJ, Vasan SS, Quinlan MM, Capurro ML, Alphey L, Bayard V et al. Deployment of innovative genetic vector control strategies: progress on regulatory and biosafety aspects, capacity building and development of best-practice guidance. *AsPac J Mol Biol Biotechnol.* 2009;17:75–85.

38. Patil PB, Alam MS, Ghimire P, Lacroix R, Kusumawathie PHD, Chowdhury R et al. Discussion on the proposed hypothetical risks in relation to open field release of a self-limiting transgenic *Aedes aegypti* mosquito strain to combat dengue. *AsPac J Mol Biol Biotechnol.* 2010;18:241–6.

39. Cayman Islands: biosafety questions asked by the Countess of Mar. London: United Kingdom Parliament; 2010.

40. Nightingale K. GM mosquito wild release takes campaigners by surprise. *Sci Dev Net*. 11 November 2010 (http://www.scidev.net/en/news/gm-mosquito-wild-release-takes-campaigners-by-surprise.html, accessed 6 November 2014).

41. Enserink M. GM mosquito trial alarms opponents, strains ties in Gates-funded project. *Science* 2010a;330:1030–1.

42. Enserink M. GM mosquito release in Malaysia surprises opponents and scientists – again. Science Insider, 27 January 2011 http://news.sciencemag.org/scienceinsider/2011/01/gm-mosquito-release-in-malaysia.html (accessed 6 November 2014).

43. Subbaraman N. Science snipes at Oxitec transgenic mosquito trial. *Nat Biotech*. 2011;29:9–11.

44. National Biosafety Board decision: application for approval for limited mark-release-recapture of *Aedes aegypti* wild type and *Aedes aegypti* genetically modified mosquitoes OX513A(My1). Putrajaya, Malaysia: Department of Biosafety, National Safety Board; 2010.

45. Liow clarifies GM *Aedes* trial. The Star Online, 29 January 2011 (http://thestar.com.my/news/story.asp?file=/2011/1/29/nation/7897715&sec=nation, accessed 6 November 2014).

46. Fong LF. Over 200 people attended GM mozzie trial talks, says IMR official. The Star Online, 31 January2011 (http://thestar.com.my/news/story.asp?file=/2011/1/31/nation/7905312&sec=nation, accessed 6 November 2014).

47. McCutcheon P. Dengue battle. Australian Broadcasting Corporation, 9 December 2010 (http://www.abc.net.au/7.30/content/2010/s3089577.htm, accessed 6 November 2014).

48. Ryan B, Sexton-McGrath K. 'Good start' to dengue eradication trials. ABC News, 2 March 2011 (http://www.abc.net.au/news/stories/2011/03/02/3152946.htm, accessed 6 November 2014).

49. Enserink M. Australia to test 'mosquito vaccine' against human disease. Science 2010b;330:1460–1.

50. De Barro PJ, Murphy B, Jansen CC, Murray J. The proposed release of the yellow fever mosquito, *Aedes aegypti* containing a naturally occurring strain of *Wolbachia* pipientis, a question of regulatory responsibility. *J Cons Protect Food Safety* 2011;6:1–10.

51. Agricultural and Veterinary Chemicals Code Act 1994. Australian Government: Canberra; 1994 (http://www.comlaw.gov.au/Details/C2004A04712, accessed 6 November 2014).

52. Murphy B, Jansen C, Murray J, De Barro P. Risk analysis on the Australian release of *Aedes aegypti* (L.) (Diptera: Culicidae) containing *Wolbachia*. Clayton, South Australia: Commonwealth Scientific and Industrial Research Organisation (CSIRO); 2010.

53. Vasan SS. Modified insects: risk analysis and public engagement (letter from the guest editor). *AsPac J Mol Biol Biotechnol.* 2010;18:237–9.

54. Secretariat of the Convention on Biological Diversity. Text of the Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety. 2011 (http://bch.cbd.int/protocol/NKL_text.shtml, accessed 6 November 2014).

55. Windbichler N, Papathanos PA, Crisanti A. Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles* gambiae. *PLoS Genet.* 2008;4:e1000291.

56. Burt A. Site-specific genes as tools for the control and genetic engineering of natural populations. *Proc Biol Sci.* 2003;270:921–8.

57. Davis S, Bax N, Grewe P. Engineered underdominance allows efficient and economical introgression of traits into pest populations. *J Theor Biol.* 2001;212:83–98.

58. Marshall JM, Pittman GW, Buchman AB, Hay BA. Semele: a killer-male, rescue-female system for suppression and replacement of insect disease vector populations. *Genetics* 2011;187:535–51.

59. Akbari O, Matzen KD, Marshall JM, Huang H, Ward CM, Hay BA. 2012. A synthetic gene drives system for local, reversible modification and suppression of insect populations. *Curr Biol.* 2012;23:671–677.

60. Ostera GR, Gostin LO. Biosafety concerns involving genetically modified mosquitoes to combat malaria and dengue in developing countries. *JAMA* 2011;305:3–391.

61. Morris EJ. A semi-quantitative approach to GMO risk-benefit analysis. *Transgenic Res.* 2011;20:1055–71.