The Cartagena Protocol and genetically modified mosquitoes

To the Editor:

The Cartagena Protocol on Biosafety[1] is the fundamental document of the United Nations on the responsible use of genetically modified (GM) organisms. Although the protocol applies to GM mosquitoes intended for disease control, its terms were negotiated primarily with concerns over the safety and trade of GM crops in mind. A sub-working group has been assigned by the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management to develop risk assessment guidelines for GM mosquitoes. Its first guidance document has recently been published following an April 2010 meeting in Ljubljana, Slovenia[2] and will be submitted to the Parties of the Protocol at their meeting next month. This is an important document outlining the potential risks of GM mosquitoes to biodiversity and human health; however, several overarching issues were considered to be beyond its scope. In this letter, I outline some of these issues and call for a broader discussion on GM mosquitoes to address their unresolved biosafety concerns.

As pointed out in the guidance document, several strategies are being developed to control vector-borne diseases using GM mosquitoes, each requiring its own risk assessment and management considerations. One strategy involves the release of genetically sterile males that, upon mating with wild females, produce unviable offspring, thus resulting in population suppression. The technology for this strategy has already been developed for *Aedes aegypti*[3]—the main vector of dengue fever—and its biosafety implications are relatively manageable because transgenes are only expected to persist in the wild for a few generations after release. Other self-limiting strategies are being developed that eliminate transgenes over subsequent generations.

Another strategy being developed involves the use of a 'gene drive system' to spread disease-refractory genes into mosquito populations, thus rendering entire populations incapable of transmitting diseases[4]. In support of this strategy, a transposable element has been observed to spread through the worldwide population of *Drosophila melanogaster* in a few decades. Progress is being made in the development of genes refractory to malaria and dengue fever, and synthetic gene drive systems are being developed for *A. aegypti* and other mosquito species. If successful, then just a few GM mosquitoes with these constructs would be capable of propagating transgenes over the entire geographical range of a species. Gene drive systems are being developed that are expected to be less capable of spreading between populations; however, this is yet to be shown in an environmental setting.

The *A. aegypti* mosquito, versions of which have been engineered to have a repressible female-specific flightless/sterile phenotype based on the use of the flight muscle promoter of Actin-4 gene.

Perhaps the most important issue inadequately addressed by the guidance document is the ability of mosquitoes engineered with gene drive systems to propagate transgenes across national borders in the absence of an international agreement. Regarding gene flow, the document expresses the need to consider "methods to reduce the persistence of the transgene in the environment" in cases where GM mosquitoes have been shown to have adverse effects. As a form of risk management, it also encourages consideration of methods for "ensuring that they [GM mosquitoes] do not establish themselves beyond the intended receiving environment." However, the acceptability of an open release of GM mosquitoes with gene drive systems that are not shown to have adverse effects is left relatively ambiguous.

A strict interpretation of the Cartagena Protocol, on the other hand, suggests that the requirements for a release of GM mosquitoes with invasive gene drive systems may be almost impossible to satisfy. The Advance Informed Agreement (AIA) procedure applies before the first environmental release of GM organisms in another country and grants the importing country the right to request the exporting country to perform a risk assessment at its own expense, part of which is to determine the likelihood of an "unintentional transboundary movement." If these movements are difficult to prevent, which is certainly the case for GM mosquitoes with invasive gene drive systems, then an environmental release is not allowed.

One way around this problem is a multilateral agreement, consistent with the protocol, which would acknowledge that any release of these mosquitoes is intentionally international and has been agreed to by the affected nations. The problem with a multilateral agreement, however, is its scale and feasibility. GM mosquitoes with invasive gene drive systems have the potential to spread transgenes over entire continents. In the context of Zambia's ban on GM food aid in 2002—during a famine that threatened hundreds of thousands of lives—a unanimous, almost worldwide agreement on GM mosquitoes seems challenging, if not impossible.

Despite this, invasive gene drive systems, such as homing endonuclease genes and

Medea elements, are being developed with the intent of driving genes refractory to malaria and dengue fever into mosquito populations. Gene drive was not an issue that was considered when the terms of the Cartagena Protocol were first negotiated and, as noted in the guidance document, the fact that mosquitoes are a vector of human disease poses "new considerations and challenges during the risk assessment process." Questions arise as to whether the risks of this technology should be weighed against the potential to control disease on a global scale. These issues must be addressed in a clear and open way, making further discussion essential.

A related issue is the exemption of GM mosquitoes in transit or destined for contained use from the AIA procedure. The AIA procedure was written, in part, with the intent of protecting developing countries against threats to biosafety due to a lack of resources to conduct their own risk assessment. Even so, during negotiations of the protocol, countries with strong biotech industries successfully argued that GM organisms in transit or containment pose negligible risks and thus the AIA procedure would restrict trade unnecessarily if applied to them. For GM mosquitoes with invasive gene drive systems, the risks are non-negligible because breaches of containment are impossible to rule out and, once released, just a few escapees could be capable of spreading transgenes on a global scale. The exemption must therefore be re-examined in these cases.

The scenario of containment is particularly relevant to GM mosquitoes because, before an open release, trials are being discussed that would take place in field cages exposed to the ambient environment in a location that the species naturally inhabits. This is an important step in a phased assessment of risks and efficiency; however, before these trials, developing countries are not entitled to request that the importing country pay for a preliminary risk assessment because the AIA procedure does not apply. This issue is not mentioned in the guidance document and was likely considered to be beyond its scope; however, the Cartagena Protocol clearly provides inadequate protection in this scenario, and further discussion is essential before field trials become a reality.

Another pressing issue hinted at in the guidance document is the inapplicability of the Cartagena Protocol to modified mosquitoes that do not fit the definition of "modern biotechnology." The protocol applies to living modified (LM) organisms developed using *in vitro* nucleic acid techniques; however, it does not apply to mosquitoes modified by other means having similar implications for biodiversity and human health.

The most noteworthy variety of non-LM mosquitoes is an *A. aegypti* line infected with the wMelPop strain of *Wolbachia,* an inherited bacterium capable of manipulating its host's reproductive biology in a manner that promotes its spread through a population. As it turns out, this *Wolbachia* infection is associated with several physiological changes beneficial for disease control, including reduced mosquito lifespan, reduced dengue viral load and reduced ability to obtain blood meals with age[5]. However, the existence of physiological changes in conjunction with invasiveness draws into question the wider implications these changes have on biosafety and highlights the fact that biosafety issues are not limited to genetic modification.

To address this issue, the guidance document states that "although the focus of this guidance is on LM mosquitoes, in principle, it may also be useful for the risk assessment of similar non-LM mosquito strategies." This is an important point; however, it is in no way legally binding. Non-LM mosquitoes are beyond the scope of the Cartagena Protocol. However, given that their biosafety implications are as serious as those for LM mosquitoes, further discussion is needed on how they should be regulated. Even-handed regulation will ensure that one strategy is not chosen over another purely for its immunity to onerous requirements.

In conclusion, the guidance document of the sub-working group represents an important first step towards incorporating the biosafety issues posed by GM mosquitoes 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 GM mosquitoes. 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 GM mosquitoes in working towards the goal of global vector-borne disease control.

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The author declares no competing financial interests.

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The *FEBS Letters*/BioCreative II.5 experiment: making biological information accessible

To the Editor:

Current publications lack structured representations of the entities and relationships they report on. As a consequence, information retrieval is hampered and much of the scientific literature is poorly accessible unless it is organized in domain-specific databases by expert curation[1]. However, manual curation is a slow process and databases lag behind, failing to cover much of the published information. The combined effort of the IMEx group deals with only ~20% of the estimated 10,000 protein interaction articles published yearly (**Supplementary Methods**). To explore new publication strategies, the *FEBS Letters* experiment asked authors to supply structured annotations for their publications that were linked to databases with the intervention of professional bio-curators[2]. The BioCreative II.5 challenge then compared these annotations provided by authors and curators to automated systems[3]. Combining these two efforts has generated the first quantitative