

Ecological Risk Assessment (ERA) for LM Mosquitoes¹

Introduction

One of the first steps in conducting an ecological risk assessment (ERA) is the identification of adverse effects.² For LM mosquitoes, this is the most critical and problematic step. The challenge is to identify potential adverse effects that are (a) feasible, (b) specific, and (c) complete, given the present fragmentary state of knowledge. To date, work in this area related to LM mosquitoes has focused primarily on identifying feasible effects, with variable regard to specificity, and no consideration of methods to ensure completeness.

Adverse effects identification is a step that helps define the scope of an ERA. The identified effects are not assumed to be significant or insignificant at this stage in the process. The identified effects are merely ones that might possibly occur, and whether they are large enough to be significant/insignificant or unacceptable/acceptable is yet undetermined. Hence it is better to err by including effects that are possible but might be thought or presumed small, rather than dismissing effects as too small to merit concern before they are actually characterized.

In addition, adverse effects identification should identify both primary and secondary ecological effects (aka direct and indirect effects).³ Some secondary effects may seem to be a logical stretch of the imagination or *a priori* quite small, but again, during the adverse effects identification step, it is more important to include such effects rather than dismiss them too early in the ERA process.

Specificity requires identification of an assessment endpoint.⁴ This involves identification of an appropriate ecological entity and attribute or that entity, the relation between the assessment endpoint and the adverse effect, one or more causal pathways between the stressor and the assessment endpoint (aka “risk hypothesis”), and a conceptual model by which the risk can be assessed. In the cases considered to date, potential effects of the released mosquitoes on other species have been considered, but only in the most general and cursory way. In no case is the other species identified by name, and no assessment endpoint is considered or proposed.

¹ D. A. Andow, Department of Entomology, University of Minnesota, St. Paul, MN, USA, dandow@umn.edu

² Adverse effects identification: Process for determining if a stressor can cause adverse effects and what those adverse effects might be. See definition for “Hazard identification.”

³ Primary effect: An effect where the stressor acts on the ecological component of interest itself, not through effects on other components of the ecosystem (synonymous with “Direct effect”).

Secondary effect: An effect where the stressor acts on supporting components of the ecosystem, which in turn have an effect on the ecological component of interest (synonymous with “Indirect effect”).

⁴ Assessment endpoint (ecological): An explicit expression of the environmental value that is to be protected, operationally defined by an ecological entity and its attributes.

There is no way to guarantee that a risk assessment is complete. However, there are methods that can be used to ensure that it is complete up to the present state of knowledge. The method that has been used extensively with LM mosquitoes is “event tree analysis.” This method starts conceptually with the release of an LM mosquito and follows a logical stream of subsequent events to identify possible things that could go wrong. Event-tree analysis is quite useful, but is known to be incomplete (for an example, see Lewis 1980). A combination of event-tree and “fault-tree” analysis (described below) has proven to identify adverse effects more completely. In addition, several more recently proposed methods extend these methods.

To complete adverse effects identification for LM mosquitoes, the following should be done: (a) a consideration of the kinds of possible adverse effects that has scientific consensus; (b) a socio-cultural process to identify environmental values of the people that expect to receive LM mosquitoes that are potentially at risk; (c) a process to specify the species and ecological processes that could be affected by the introduction of the mosquitoes; (d) a process to link conceptually the identified environmental values to the introduction of the mosquito stressor; (e) a process to screen rapidly the identified possible adverse ecological effects to allow greater concentration on the more serious ones.

Kinds of Adverse Effects

Although there has long been a scientific consensus that all of the kinds of adverse effects⁵ associated with environmental release of genetically engineered species are known (NRC 1987; Snow and Moran-Palma 1997; NRC 2002; Snow et al. 2005), this has not been carefully evaluated for LM mosquitoes. Hence it is reasonable to consider whether the kinds of adverse effects previously identified for GEOs are applicable to LM mosquitoes. The kinds of adverse effects that have been identified for GEOs are: A) adverse effects on ecological species, habitats and ecosystem processes, B) adverse effects associated with gene flow, and C) evolutionarily mediated adverse effects. I will identify the kinds of potential adverse effects associated with LM mosquitoes in general terms, numbering them from A1 to C3, where the letter corresponds to the major categories of potential adverse effects and the numbers to subtypes.

Stressor identification

In all cases considered here, there are several potential stressors⁶ associated with the release of mosquitoes. These are:

- the released LM mosquitoes,
- the associated production facilities and
- infrastructure for releasing LM mosquitoes, and/or
- effects of the released LM mosquitoes on the target mosquitoes (LM mosquito as a secondary stressor)⁷

⁵ Adverse ecological effect: Change that is considered undesirable because it alters valued structural or functional characteristics of ecosystems or their components. An evaluation of adversity may consider the type, intensity, and scale of the effect as well as the potential for recovery.

⁶ Stressor: Any physical, chemical, or biological entity that can induce an adverse effect

In the remainder of this discussion, I will refer to the “released mosquito” as the stressor, but in all cases, the reader should substitute this list of four possible stressors as possibilities for ERA. Certainly, during the early stages of LM mosquito product development, efforts at ecological risk assessment might focus on the released mosquitoes and the effects of the release on the target mosquitoes, rather than the associated production and release infrastructure. This is because the specific infrastructure needs will be determined during the implementation stage of the projects, when an ERA focusing on this can be developed most efficiently, and because the ERA approach might be quite different than that for the LM mosquito, perhaps following a HACCP model.

A) Adverse effects associated with ecological species, habitats and ecosystem processes

New or more vigorous pests, especially adverse effects on human health. (1) The released mosquitoes may not function as expected. Gene silencing or production failures could result in the release of non-sterile or competent mosquitoes and thus increasing the vector population or disease transmission. (2) The released mosquitoes could transmit another disease more efficiently. Such diseases might include yellow fever, chikungunya, and others. (3) Suppression of the target mosquito might enable another vector species to increase, resulting in higher levels of the target disease or a new disease in humans. These include other mosquitoes and vectors of other diseases, such as schistosomiasis. (4) The released mosquitoes might become nuisance pests or may be perceived to cause problems to human health. (5) The released mosquitoes might cause other pests to become more serious, including agricultural pests and other pests of other valued human activities.

Harm to or loss of other species. (6) The released mosquitoes might cause other valued non-pest species (for instance fishes) to become less abundant. These include species of economic, cultural, and/or social importance, such as wild foods, iconic species, and endangered species. Ecological effects might result from competitive release if the target mosquito is reduced or from trophic consequences of species that rely on mosquitoes for food during some specific time of the year. Effects might also occur if the target mosquito was also transmitting a disease to another animal species, if the released mosquitoes transmit a disease of another animal species more efficiently, or if a vector of an animal disease was released from ecological control by the reduction of the target mosquito. Sterile interspecific matings between released LM mosquitoes and other mosquito species could disrupt the dynamics of these other species, leading to harm or loss of valued ecological species.

Disruption of ecological communities and ecosystem processes. (7) The ecological communities in the ephemeral, small aquatic habitats occupied by the mosquitoes presented targeted with LM mosquitoes are unlikely to be greatly disrupted beyond the possibilities already addressed above under “harm to or loss of other species.” However, if the released mosquitoes were to inhabit more natural habitats, such as tree-holes, disruption of the associated community should be considered. (8) The released mosquitoes might degrade some valued ecosystem process. This might include processes such as pollination or support of normal ecosystem functioning. These processes are often referred to as ecosystem services (Costanza et al. 2000, MEA 2005), but the valued processes may be culturally or socially specific.

⁷ Secondary stressor: An entity that is directly or indirectly affected by the primary stressor and itself can induce an adverse effect.

B) Adverse effects associated with gene flow

Gene flow focuses on the movement of the novel trait. This can occur via movement of the released organism, sexual transmission of genetic elements, or independent movement of the genetic elements. Whether gene flow occurs and what undesirable effects it might have depends on various factors, such as the technology used, the trait or traits carried by the mosquitoes, the receiving environment, etc.

Spread of released organism and sexual transmission. Some LM mosquitoes are being designed to spread a trait rapidly through the target mosquito population. For *Anopheles gambiae*, the trait may be expected to spread throughout the *A. gambiae* species complex. In these cases, intraspecific or intraspecies-complex gene flow will not be expected to cause any adverse effects beyond those mentioned in the other sections, A and C. (1) Other LM mosquito technologies are not expected to spread in the target mosquito population or are designed to be self-limiting. The potential for unexpected spread of the released trait should be considered for these by focusing on the ways any failsafe mechanism to limit spread could break down. (2) Interspecific gene flow should be considered for all of the technologies. Mosquitoes, like other insects, typically have strong reproductive isolating mechanisms that will not allow interspecific gene flow. Identifying the key reproductive isolating mechanisms and the conditions leading to their breakdown could be a focus of this assessment. In addition, the fitness conferred by the trait or symbiont, and the size and frequency of the release also determine the rate of spread of the trait (Haygood et al. 2003).

Independent movement of genetic elements. This is commonly referred to as horizontal gene flow, which is the movement of genetic information from one organism to another through means other than sexual transmission. (3) The risk associated with horizontal gene flow may be small, but should still be considered. Breakdown of the control and specificity of the gene drive mechanism might be an initial focus for this assessment. The risk of horizontal gene flow in LM mosquitoes without a gene drive system might be expected to be much smaller.

C) Evolutionarily mediated adverse effects

Any strong ecological effect also exerts an evolutionary selection pressure. The main evolutionary effects are those that could result in a breakdown in the technology and the resumption of previous disease levels. Other evolutionary effects could be hypothesized, but they would first require the occurrence of some adverse effect on a species, community or ecosystem effect, and therefore consideration of secondary evolutionary effects can be postponed until such effects are identified and found to be significant. In general, evolutionary effects should consider three possible entities:

Mosquito. (1) The mosquito vector might evolve to avoid population suppression, regain competency, or acquire new or enhanced competency of another disease agent.

Trait or symbiont. (2) The trait may evolve to lose effectiveness.

Disease agent. (3) The disease agent might evolve to become more virulent, overcome non-competency mechanism, or acquire new vector species.

The identification of the kinds of possible adverse environmental effects of LM mosquitoes is extremely valuable, because by identifying all of the kinds of adverse effects, we ensure that the identification of adverse effects can be complete as long as they are specifically identified for a particular LM mosquito.

Identification of adverse effects

Once the kinds of possible adverse effects are clarified, the next step is to identify a specific and complete list of potential adverse effects at a more detailed level for a particular LM mosquito (LM trait, mosquito species, and intended environment for release).

To date, the work completed on identifying adverse ecological effects of LM mosquitoes is incomplete, and when effects are identified, they are often not sufficiently specified to be amenable to assessment (Table 1). However, this does not yet pose a serious problem, because all of the technologies being considered are early enough in the development process that there is sufficient time to rectify the situation. A few cases have completed outstanding adverse effects identification. These include the potential increase in the target disease in the event that fsRIDL does not perform as expected (A1), and the analysis of evolutionary effects associated with the HEG trait (C2). However, it should be noted that an ERA does not necessarily have to rise to the level of rigor of these two examples, because ERA uses a weaker evidentiary standard (weight of evidence) than that used in normal scientific activity.

Three major problems related to ERA need to be addressed during the early phases of development so that ERA can be completed in a timely manner:

- 1) The approaches and methodologies for ERA should become more uniform.***
- 2) Most of the adverse effects need to be identified at a greater degree of specificity.***
- 3) Although completeness is elusive, methods that deliberately help ensure completeness and avoid unconscious disciplinary bias need to be actively sought and employed.***

The greatest lack of specificity and completeness is associated with effects A5-A8 (Table 1). Addressing these is by far the greater challenge, and I will spend most of the rest of this review suggesting how to address this issue.

In addition, except for HEG, the evolutionary implications of the other technologies have not been evaluated (C1-C3). The modeling approach used by Deredec et al. (2008) is an exemplar that should be adapted to analyze the other technologies.

Finally, work on the other kinds of adverse effects can be developed in more detail, but the approaches are more straightforward and the gaps should be relatively easy to fill. For example, to identify potential vectors that could be released from competition with the target mosquito (A3), one needs to know the vector species that share larval habitat with the target species, and vector species that share adult resources (resting sites, hosts, oviposition sites, etc.) with the target species. Hence, I will not address these any further here, even though they should be addressed.

Environmental Values Potentially at Risk

A) Identifying relevant environmental values

Adverse ecological effects are undesired changes to valued ecological entities (see definitions). When experts are unable to identify the adverse ecological effects, or the identified effects seem incomplete, then one useful approach is to start by identifying relevant environmental values held by a society. Adverse effects associated with A5-A8 inclusive are partially defined by reference to the ecological entities related to valued components of the environment. Thus, to specify and complete ERA for these potential adverse effects, it would be useful to determine the relevant environmental values of the societies where mosquito releases might occur.

These values might be expressed by individuals in very general terms, such as a love for open spaces, a love of the outdoors, or a love of the forest, and in specific terms, such as a desire for a white Christmas or a fear of tornados. These are all stated preferences. In addition, values are expressed in the choices people make, such as visiting a lake instead of a sporting event. These are realized preferences.

Values are also expressed socially by the institutional, political and informal networks that engage the people in a society. These include laws, such as the Endangered Species Act, treaties, such as the Convention on Biological Diversity, and many other associations related to social governance. But they are also expressed through the strength of informal social networks. For example, the existence of the Audubon Society, the WWF, and Greenpeace embody the realization of strong informal social networks oriented around environmental values, with each representing a different constellation of values.

Values are also expressed culturally. In this way, they represent some of the underlying, unstated, and sometimes unrecognized bases of society. For example, Americans in the US hold the American bald eagle in almost reverential regard, and surprisingly, the monarch butterfly is also an important cultural symbol. In much of south and southeast Asia, the Bohdi tree, *Ficus religiosa*, is held in high regard because it is the tree under which Siddhartha Gautama became enlightened, and is thus a symbol for happiness, prosperity, longevity and good luck.

Values can be elicited using directed psychological, sociological, anthropological and economic research in the communities where initial releases of mosquitoes might occur to develop a list of the major environmental values held by those populations. These lists should include valued economic activities that rely on the environment, the species with economic, cultural and conservation value, valued habitats, and valued ecosystem functions, such as the air purifying capacity of forests, pollination services, specific processes that maintain ground and surface water quality, etc. The purpose of these activities is to identify significant environmental values. It is not to establish a link between mosquitoes and those values, as developing such links requires different methods and expertise.

B) Specifying adverse effects on species, communities and ecosystem processes

Once the relevant environmental values have been identified, a multi-disciplinary group can convene to make conceptual links between the environmental values and specific adverse effects

on species, communities and ecosystem processes. The output should be two sentences to a paragraph that specifies a species, ecological community or an ecosystem process, an attribute or several attributes⁸ of that ecological entity⁹ that measures the degree of adversity of ecological change, and the way this change relates to the environmental value. Multiple entities and attributes may be associated with a single environmental value. These identified entities and attributes can be used as assessment endpoints.¹⁰

C) Linking environmental values to stressor

Following the specification of potential assessment endpoints, the same or similar group of people and expertise should link the assessment endpoints to the potential stressors. This linkage should comprise a series or network of possible causal connections from the primary stressor to the assessment endpoint, either directly or indirectly. Several methods should be used to develop the linkages and avoid bias and anchoring, including qualitative fault tree analysis (Haines 1998, Hayes 2002a), infection modes and effects analysis (Hayes 2002b), and hierarchical holographic modeling (Hayes et al. 2004). Structured approaches leading to agreement and simplification should be used, and may include using Bayesian nets (Fenton and Neil 2001), multicriteria decision analysis (Fenton and Neil 2001, Dodgson et al. 2009), fuzzy logic (Bojorquez et al. 2002, Shepard 2005) and other methods. The output will be specific risk hypotheses that can be prioritized and assessed.

D) Screening risk assessment

A screening risk assessment is performed with few data and many assumptions to identify exposures and effects that should be evaluated more carefully (Suter 2007). The purpose is to evaluate rapidly the many possible adverse effects to separate those that are small enough or unlikely enough from those that may be large enough or likely enough to be significant risks. Typically a screening risk assessment makes many worst-case assumptions to guard against type II errors. Screening risk assessments are distinguished from definitive risk assessments, which aim to estimate, describe and interpret risk. Both screening and definitive risk assessments may be qualitative, semi-quantitative, or quantitative, however, many screening risk assessments rely on expert judgment, and are necessarily qualitative.

Once the possible adverse effects have been appropriately identified (as discussed above), the next logical step is to conduct a screening risk assessment to determine which if any of these identified possible adverse effects require definitive assessment.

The standard screening methods are based on ecotoxicological methods, such as hazard quotient (Suter 2007), and may not be applicable for screening effects associated with released mosquitoes. Several alternative approaches are possible. 1) Show that the magnitude and

⁸ Attribute: A quality or characteristic of an ecological entity.

⁹ Ecological entity: A general term that may refer to a species, a group of species, an ecosystem function or characteristic, or a specific habitat.

¹⁰ Assessment endpoint (ecological): An explicit expression of the environmental value that is to be protected, operationally defined by an ecological entity and its attributes. For example, salmon are valued ecological entities; reproduction and age class structure are some of their important attributes. Together “salmon reproduction and age class structure” form an assessment endpoint.

probability of the worst-case risk is a negligible change to the present. 2) Show that worst-case exposures to the released mosquitoes are orders of magnitude too small to cause a significant risk (magnitude and probability of the adverse effect). This is an adaptation of the hazard quotient method. If necessary a third approach can be used, 3) involving stakeholders to judge or rank the significance of potential adverse effects. The results of this third approach may be highly site specific.

E) Developing risk management options

At the same time that the risk assessment activities are going on, methods development for risk management should be conducted. Monitoring methods must be simple, inexpensive and accurate. The following monitoring needs should be considered:

- Monitoring released mosquitoes and the GE trait during contained use and open environmental release;
- Monitoring the potential evolutionary breakdown of the mosquito technology.
- Monitoring efficacy and effectiveness of mosquito technology.
- Monitoring of stakeholder perceptions of the progress of the technology.

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Appendix: Definitions

Adverse ecological effect: Change that is considered undesirable because it alters valued structural or functional characteristics of ecosystems or their components. An evaluation of adversity may consider the type, intensity, and scale of the effect as well as the potential for recovery. (USEPA 1998) n.B., the change occurs to an attribute(s) of a specified ecological entity; this attribute and entity may or may not be the same as the assessment endpoint.

Adverse effect: Change that is considered undesirable because it alters valued characteristics of the environment or human health. An evaluation of adversity may consider the type, intensity, and scale of the effect as well as the potential for recovery. (adapted from USEPA 1998)

Adverse effects identification: Process for determining if a stressor can cause adverse effects and what those adverse effects might be. Synonymous with “Hazard identification.”

Assessment endpoint (Ecological): An explicit expression of the environmental value that is to be protected, operationally defined by an ecological entity and its attributes. For example, salmon are valued ecological entities; reproduction and age class structure are some of their important attributes. Together “salmon reproduction and age class structure” form an assessment endpoint. (USEPA 1998)

Attribute: A quality or characteristic of an ecological entity. An attribute is one component of an assessment endpoint. (USEPA 1998)

Definitive risk assessment: A risk assessment performed with all relevant data and information to characterize the risk for a given adverse effect or assessment endpoint. (Suter 2007)

Direct effect: See “Primary effect.”

Ecological entity: A general term that may refer to a species, a group of species, an ecosystem function or characteristic, or a specific habitat. An ecological entity is one component of an assessment endpoint. (USEPA 1998)

Hazard assessment: 1. Evaluation of the intrinsic effects of a stressor (USEPA 1979; synonymous with “Screening risk assessment”). 2. Calculating a hazard quotient by comparing a toxicologic effects concentration with an exposure estimate (SETAC, 1987; synonymous with “Safety assessment”). (from USEPA 1998)

Hazard identification: Process for determining if a stressor can cause adverse effects and what those adverse effects might be. Synonymous with “Adverse effects identification.”

Hazard: 1. Potential for a stressor to cause human illness or injury (synonymous with “Adverse effect”). 2. The inherent toxicity of a chemical. Hazard identification of a given chemical is an informed judgment based on verifiable toxicity data from animal models or human studies (see general definition of “Hazard identification”).

Indirect effect: See “Secondary effect.”

Primary effect: An effect where the stressor acts on the ecological component of interest itself, not through effects on other components of the ecosystem (synonymous with “Direct effect;” compare with definition for secondary effect). (from USEPA 1998)

Receptor: The ecological entity exposed to the stressor. (from USEPA 1998) See also definition for secondary receptor.

Risk: Probability that a specified adverse effect of a given magnitude occurs or the set of probabilities associated with all possible magnitudes of a specified adverse effect.

Screening risk assessment: A risk assessment performed with few data and many assumptions to identify exposures and effects that should be evaluated more carefully for potential risk. (Suter 2007)

Secondary effect: An effect where the stressor acts on supporting components of the ecosystem, which in turn have an effect on the ecological component of interest (synonymous with “Indirect effect;” compare with definition for “Primary effect”). (from USEPA 1998) n.B., tertiary, quaternary and higher order effects can also occur; these are all subsumed within the definition of secondary effect.

Secondary receptor: An entity that is indirectly exposed to the primary stressor.

Secondary stressor: An entity that is directly or indirectly affected by the primary stressor and itself can induce an adverse effect.

Stressor: Any physical, chemical, or biological entity that can induce an adverse effect (synonymous with “Agent” and “Primary stressor”). (EPA 1998) See also definition for secondary stressor.

Appendix References

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Table 1. An evaluation of present status of adverse effects identification for the kinds of potential adverse effects. Consideration of F = feasibility; S = specificity; C = completeness of identified adverse effects. Evaluation criteria: U = unspecified or very incomplete; P = partially specified or partially complete; W = well specified or very complete.

Kind of Potential Adverse Effect	<i>Aedes aegypti</i> RIDL			Guidance for Contained Field Trials of LM Mosquitoes with Novel Gene Drive System	
	F ¹	S	C	F ²	S
A1. Technology failure	1 Male starts to bite 2 Biting period extended 4 LM mosquito lives longer 5 Wild females more aggressive after mating with LM males 6 LM mosquitoes better able to escape predators 12 Stability of gene construct 13 Increased mosquito population 14 Increased mosquito size 15 Increased fecundity of wild females 18 Larvae survive without water 20 More blood per bite 21 Tetracycline allergy 24 Accidental release 26 Increase in human allergy			Increased transmission of target pathogen Increase in fitness Increase in biting rate Undesired effects on mosquito population dynamics Predicted rate of spread Predicted equilibrium frequency of transgenic trait	P
A2. New disease	10 Vector capacity increased 11 Infection with multiple viruses			Increased transmission of other pathogens	U
A3. Vector release	19 Reduced survival of target 30 Increase in <i>Ae. albopictus</i>				
A4. Nuisance or perceived risk	7 LM males mate multiply 8 LM male self-replicates 29 Work hazard			Increase in nuisance behavior	U
A5. Other pests					
A6. Harm or loss of species	17 Increased host range 23 Effect on ecosystem 25 Target becomes endangered				
A7. Community	28 Dead larvae				
A8. Ecosystem	31 Change water and soil quality			Persistence of transgenic material in environment	U
B1. Intra flow					
B2. Inter flow	3 Cross mating with other mosquito species			Horizontal gene transfer to another species	U
B3. Horizontal flow	22 Gene transfer to humans 27 Gene transfer to plants				
C1. Evolution, mosquito	9 LM males with higher resistance to insecticides 16 Become heat resistant 17 Increased host range			Decreased susceptibility to other control measures	P
C2. Evolution, trait					
C3. Evolution, disease agent					

¹ Beech et al., manuscript distributed publicly at WHO meeting, May 2009

² Benedict et al. 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): 127-166.

³ Need also to consider the possibility that the technology does not work as planned or breaks down

⁴ Need to consider more species than just *Aedes albopictus*

⁵ Only considers perception of working in a GE mosquito production facility

⁶ No species are specified, and only consideration of consumers of mosquitoes, and endangerment of the target mosquito species

⁷ Community not specified, effect not specified

⁸ Only consider generalized changes to water and soil quality

⁹ Need to consider other *Aedes* species and how reproductive isolating mechanisms may break down

¹⁰ Only humans and plants considered

¹¹ Only ancillary evolutionary effects considered