Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values

DETAILS

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Gene Drives on the Horizon:

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Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct

Board on Life Sciences

Division on Earth and Life Studies

The National Academies of
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Preface

Historians of science and engineering illuminate how discoveries related to theory, observation, and technology change our understanding of the natural world and the ways in which we interact with the organisms around us. Occasionally, the pace of discovery in a particular research area is so rapid it is impossible to miss. If the current pace of change in general genetics is thrilling, the pace of change in gene drive research is breathtaking. Not surprisingly, the depth, breadth, and practical implications of scientific advances in gene drive research are simultaneously raising many challenges at the interface of science and society.

The National Institutes of Health and the Foundation for the National Institutes of Health asked the Board on Life Sciences of the National Academies of Sciences, Engineering, and Medicine to convene a consensus committee to summarize current understanding of the scientific discoveries related to gene drives and their accompanying ethical, legal, and social implications.

This report reflects the committee's consensus conclusions regarding the state of the science and expectations for responsible research. The committee's analyses are based on reviews of the multidisciplinary literature, interviews of experts, and presentations from natural and social scientists working at the leading edges of research on gene drives and related technologies. Appropriate for such a task, the committee's 16 members have diverse interdisciplinary expertise and a range of backgrounds across the natural and social sciences, ethics, and the law. The committee often had to re-examine fundamental aspects of genetics, population biology, probability, public policy, and the law in order to understand the full scope of gene drive research and its effects. To ensure that the audience has a common understanding of the scientific, social, and regulatory knowledge essential to responsible research with gene drives, the report also outlines some of these fundamentals before moving to the complex picture we ultimately describe.

This report would not have been possible without the exceptional contributions of the Academies staff members: Keegan Sawyer, Audrey Thévenon, Robin Miller, Nancy Huddleston, and Frances Sharples. Angela Kolesnikova provided the committee with outstanding logistical support. We acknowledge gratefully all of their efforts.

A special thanks goes out to our colleagues on the committee for their thoughtful review and analysis of an enormous amount of information some of which changed on a daily or weekly basis as new discoveries were made. It was an honor to work with all of them.

James P. Collins, *Co-Chair*
Elizabeth Heitman, *Co-Chair*
Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct

Acknowledgment of Reviewers

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this report:

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Kenneth Oye, Massachusettes Institute of Technology
Ronald Sandler, Northeastern University

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the report's conclusions or recommendations, nor did they see the final draft of the report before the release. The review of this report was overseen by Stephen Barthold, University of California, Davis, and Barbara Hansen, University of South Florida. They were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Summary

Scientists have studied gene drives for more than 50 years. The development of a powerful genome editing tool in 2012, CRISPR/Cas9[1] led to recent breakthroughs in gene drive research that built on that half century's worth of knowledge, and stimulated new discussion of the potential applications and implications of gene drive technologies. Just prior to the beginning of this study and since the committee was first convened, scientists published four proofs of concept—one in yeast, one in fruit flies, and two in different species of mosquitoes—that demonstrate the successful development gene drives in the laboratory, at least in these organisms. Proposed applications for gene-drive modified organisms for basic research, conservation, agriculture, public health and other purposes will likely continue to expand as gene editing tools become more refined. Gene-drive modified organisms are on the horizon.

The fast moving nature of this field is both encouraging and a concerning. While gene-drive modified organisms hold promise for addressing difficult to solve, persistent challenges, such as the eradication of vector-borne diseases and the conservation of threatened and endangered species, these proposed applications are based on limited proof-of-concept studies. The presumed efficiency of gene-drive modified organisms may lead to calls for their release in perceived crisis situations, before there is adequate knowledge of their ecological effects, and before mitigation plans for unintended harmful consequences are in place.

Responding to this fast moving field, the National Institutes of Health (NIH) and the Foundation for the National Institutes of Health (FNIH)[2] asked the National Academies of Sciences, Engineering, and Medicine to convene a committee with a broad range of expertise to summarize the scientific discoveries related to gene drives and considerations for their responsible use.

Proof-of-concept in a few laboratory studies is not sufficient in and of itself to support a decision to release gene-drive modified organisms into the environment. Laboratory and field research is needed to refine CRISPR/Cas9-based gene drives and other gene drive mechanisms, and to understand how gene drives might work under different environmental conditions and in a wide variety of organisms. The considerable gaps in knowledge about potential off-target (within the organism) and non-target (in other species or the environment) effects necessitate a collaborative, multidisciplinary approach to research, ecological risk assessment, development of public policy, and decision making for each proposed application of a gene drive technology. General principles to guide responsible practices for gene drives from the laboratory setting through to field release and monitoring are embedded as recommendations throughout the report.

STATE OF THE SCIENCE OF GENE DRIVES

Gene drives are systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced (see Figure S-1). Thus, the result of a gene drive is the preferential increase of a specific *genotype*, the genetic makeup of an organism that de-

[1]CRISPR (Clustered regularly-interspaced short palindromic repeats) are segments of bacterial DNA that, when paired with a specific guide protein, such as Cas9 (CRISPR associated protein 9), can be used to make targeted cuts in an organism's genome.

[2]This study was sponsored by the National Institutes of Health and the Foundation for the National Institutes of Health, and the National Academy of Sciences Biology Fund. The Defense Advanced Research Projects Agency and The Bill and Melinda Gates Foundation provided support to the NIH and the FNIH, respectively for this study.

termines a specific *phenotype* (trait), from one generation to the next, and potentially throughout the population.

A wide variety of gene drives occur in nature that can cause genetic elements to spread throughout populations to varying degrees. Researchers are studying how to harness such natural mechanisms (e.g., transposable elements, homing endonucleases, and meiotic drive) to develop gene-drive modified organisms. Preliminary evidence suggests that gene drives developed in the laboratory with CRISPR/Cas9 could spread a targeted gene through nearly 100% of a given population of yeast, fruit flies, or mosquitoes.

The development of CRISPR/Cas9 as a genome editing tool has spurred biologists to propose a range of applications for gene drives to solve various public health, agricultural, conservation, and other challenges where solutions are limited or entirely lacking (see Table S-1). Most research to date is focused on controlling or altering organisms that transmit infectious diseases to humans, such as mosquito vectors of dengue, malaria, Zika, and chikungunya.

A gene drive that alters the female mosquito's ability to become infected with the malaria parasite, or prevents parasite development within the mosquito, could block malarial transmission without affecting mosquito populations. In November 2015, researchers demonstrated that CRISPR/Cas9 can be used to create a gene drive to spread anti-*Plasmodium* genes in populations of a malaria-carrying mosquito, *Anopheles stephensi* (Gantz et al., 2015). However, the system transmits the drive construct at Mendelian frequencies in some instances, suggesting that this valuable proof-of-concept needs further modification and research before field release (Gantz et al, 2015). Alternatively, a gene drive that alters the fitness of the female mosquito could result in reducing vector populations over time. In December 2015, researchers demonstrated that CRISPR/Cas9 can be used to create a gene drive that causes sterility in female *Anopheles gambiae* mosquitoes (Hammond et al., 2016).

It is important to note that, until proven otherwise, cell types and species are expected to differ in their capacity to carry a gene drive, and therefore the effects and efficacy of gene drives will be largely species-dependent.

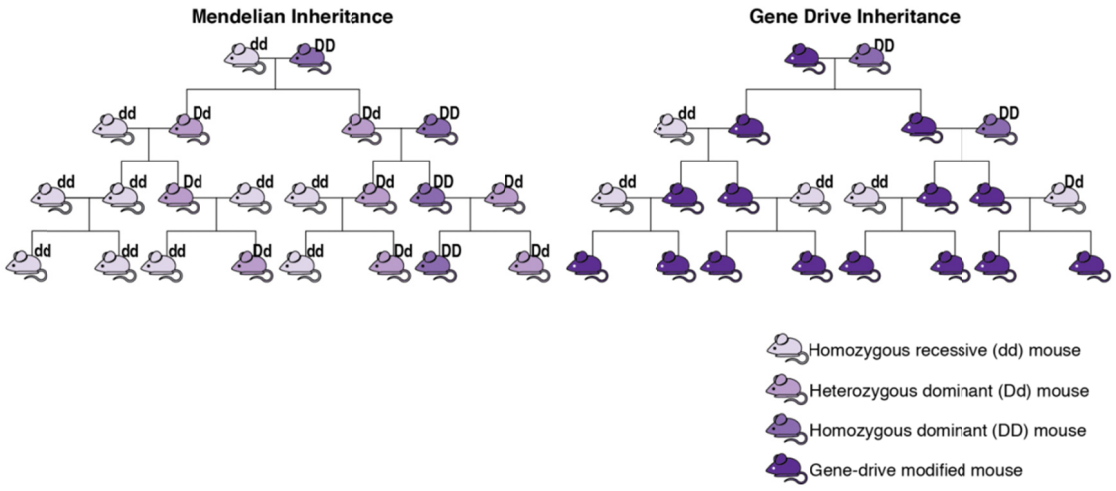
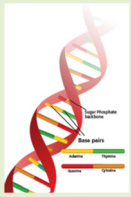


FIGURE S-1 An Idealized Illustration of Mendelian Inheritance versus Gene Drive Inheritance. Gene drives are often described as an exception to the conventional rules of inheritance first described in 1866 by a monk named Gregor Mendel. Under Mendelian inheritance (left), offspring have a 50% chance of inheriting a gene (d or D). With a gene drive (right), the offspring will almost always receive the targeted genetic element (shown in dark purple), the end results of which is preferential increase of a specific *genotype*. In this idealized illustration, the targeted genetic element is eventually present in 100% of the population, although this may not always occur. The number of generations and amount of time for a selfish genetic element (DNA sequences where inheritance is biased in their favor) to spread throughout a population will vary depending on the drive mechanism, the species, and a variety of environmental conditions.

TABLE S-1 Potential Applications for Gene Drive Research

Public Health  ***Aedes aegypti*** Image Source: US Centers for Disease Control and Prevention	• Control or alter organisms that carry infectious diseases that affect humans, such as dengue, malaria, Chagas, and Lyme disease • Control or alter organisms that directly cause infection or disease, such as Schistosomiasis • Control or alter organisms that serve as reservoirs of disease, such as bats and rodents
Ecosystem Conservation  ***Hemignathus munroi*** **('Akiapōlā'au honeycreeper)** Image Source: US Department of Fish and Wildlife Service	• Control or alter organisms that carry infectious diseases that threaten the survival of other species • Eliminate invasive species that threaten native ecosystems and biodiversity • Alter organisms that are threatened or endangered.
Agriculture  **Fruit damage from spotted wing drosophila infestation** Image Source: US Department of Agriculture	• Control or alter organisms that damage or carry crop diseases • Eliminate weedy plants that compete with cultivated crops
Basic Research  **DNA Double Helix** Image Source: National Institutes of Health	• Alter model organisms to carry out research on gene-drive function and effects, species biology, and mechanisms of disease

ECOLOGICAL AND ENVIRONMENTAL CONSIDERATIONS

Research on the molecular biology of gene drives has outpaced research on population genetics and ecosystem dynamics, two fields of study whose perspectives are essential to determining the efficacy of gene drives and their biological and ecological outcomes. There are considerable gaps in knowledge regarding a gene drive's effectiveness, both on the target organism and the environment, over time and across diverse genetic backgrounds. It is also essential to consider how gene drives will propagate throughout a population and affect not only the target species, but its entire ecological community. Key factors that influence the propagation of gene drives include the following:

- The evolutionary "fitness" of individuals carrying the gene drive—that is, their ability to produce fertile offspring—as compared to individuals not carrying the gene drive.
- The "conversion rate," which describes how the gene drive is passed to subsequent generations when one parent carries the gene drive and the other does not.
- "Gene flow," which describes how the gene drive moves between different populations of the target species.

- "Horizontal gene transfer," or the potential for gene drives to move from the target species into entirely different species.

These four factors interact in complex ways. Improved modeling capabilities and more empirical evidence would enhance our ability to understand and predict how gene drives might propagate through populations. It is also vital to consider how changes in a species' population size or distribution that are caused by a gene drive might reverberate through the ecosystem as a whole. Pertinent community dynamics and ecological factors to consider include the following:

- What is the species' role in its community?
- Are there other species that would fill a similar ecological niche in the community if the target species were to disappear?
- Is there a tipping point at which the community may change rapidly from one configuration to another, and could the gene drive lead to such a tipping point?
- How might a dramatic change in the population of the target species affect other species with which it has coevolved?
- Could the target species develop mechanisms to neutralize the gene drive (e.g. evolve resistance), and how might that dynamic affect others in the ecological community?

Generally speaking, a gene drive would likely be most harmful to an ecosystem if it is released in a native keystone species, but potentially less impactful if deployed in an invasive species for which there is a native ecological equivalent, thus containing the impacts to a relatively small part of the food web. Unintended consequences should be considered, especially in regard to the risk of horizontal gene transfer. In order to address knowledge gaps, gene drive research will require the convergence of multiple fields of study including molecular biology, genome editing, population genetics, evolutionary biology, and ecology.

CHARTING HUMAN VALUES

Questions about gene drives rest on values at every step, from whether, why, and how research should be conducted to whether and where a gene-drive modified organism should be released into the environment. Three broad categories of concern were identified and explored:

- the potential benefits and harms of gene drive research for people,
- the potential impact of gene-drive modified organisms on the environment, and
- the use of gene drives and who will make decisions about them.

The potential benefits and harms of gene-drive modified organisms will be central in deciding whether to allow field testing or open environmental release. Some of the fundamental reasons to conduct gene drive research include widely shared commitments to fighting human disease, promoting human welfare, and protecting and restoring the natural environment. A hypothetical example is the potential development of a gene drive that prevents mosquitoes from transmitting dengue, a virus that occurs predominately in urban environments throughout the tropics, could save many lives.

On the other hand, some gene-drive modified organism might pose harm to humans. One hypothetical example is a mosquito modified so that it could not host the dengue virus that becomes more susceptible as a host to another virus. Deciding whether to go forward with environmental release of a gene-drive modified organism will require a reasonable level of assurance that the possible harms have been identified and studied and that they are outweighed by the potential benefits.

A potential environmental benefit is that a gene drive may be less harmful than alternative solutions to a problem. For example, a gene drive to suppress non-native rodent populations on remote islands could reduce the need for alternative forms of control such as the use of rodenticides. The cost of admin-

istering rodenticides is estimated to be in the millions of dollars and rodenticides may also harm non-target species.

Nonetheless, because gene-drive modified organisms are intended to spread in the environment, there is a widespread sense among researchers and commentators that they may have harmful effects for other species or ecosystems. For example, using a gene drive to suppress a non-native weed population may lead to unexpected consequences, such as the loss of habitat for native species or even the establishment of a second, more resilient invasive species. Assessments of the environmental harms of a proposed release will require careful, case by case analysis

Values related to human welfare and environmental harms will be weighed in developing public policy guidelines, some of which may constrain research on gene drives or the release of gene-drive modified organisms into the environment. Such guidelines will require integrating precautionary measures into the research process and the assessment of potential benefits and harms. Precautionary measures can provide opportunities to gather further information and revisit decisions about how to proceed with a gene-drive technology, but, at the same time, not hinder research progress.

Perspectives on the place of human beings in ecosystems and their larger relationship to nature—and their impact on and manipulation of ecosystems—have an important role in the emerging debate about gene drives. The increased power for human beings to alter wild species and perhaps to eliminate them, thereby altering the shared environment—will be intrinsically objectionable to some people. Proposals to use gene drives in ways that might lead to the extinction of species or significantly alter the environment will require especially careful review.

When selecting sites for field trials or environmental releases of gene-drive modified organisms, it is important to consider the values of researchers and the affected publics, and their understanding of the balance of potential benefits and harms. Approaches to ensure that communities participate meaningfully in decision-making about the use of gene-drive modified organisms will be essential, particularly in low and middle-income countries where power differentials may preclude such participation.

PHASED TESTING AND SCIENTIFIC APPROACHES TO REDUCE GENE DRIVES' POTENTIAL HARMS

Before field testing or environmental release of gene-drive modified organisms, it is crucial to establish a rich understanding of the target organism, its relationship with its environment, and potential unintended consequences. A phased testing pathway, such as the one outlined by the World Health Organization (WHO) for testing genetically modified mosquitoes, can facilitate a precautionary, step-by-step approach to research on gene drives.[3] Each step in such a pathway promotes careful study and evaluation, includes checkpoints to determine whether and when research should move to the next phase, and provides vital data to inform and enhance the effectiveness of other phases.

In contrast with other genetic modification techniques, which are typically designed to minimize inheritance or transmission of altered genetic elements, the goal of a gene drive is to rapidly spread genetic information throughout a population**.** This makes it especially important to minimize the potential for unintended consequences. Reducing the potential for unintended consequences will require a combination of confinement and containment strategies.

When developing confinement and containment strategies, consideration should be given to their benefits, costs, and weaknesses. For example, adding a visible marker to help identify gene-drive modified organisms in some cases could have negative consequences for the organism, which should be weighed against the benefits of this strategy. It is particularly imperative to use caution when considering the development of a "reversal drive"—a gene drive designed to mitigate the unintended consequences of

[3]WHO (World Health Organization). 2014. The Guidance Framework for Testing Genetically Modified Mosquitoes. World Health Organization, Programme for Research and Training in Tropical Diseases [online].

another gene drive—as it may be impossible to effectively employ this strategy without off-target effects or to fully redress ecological and environmental effects from the original gene drive.

After release into the environment, a gene drive knows no political boundaries. Thus, it is desirable to expand the intellectual capital of governing bodies and research capacity of relevant institutions around the world to facilitate appropriate engagement in governance, research, and collaboration pertaining to gene drives. In particular, this includes building long-term relationships with scientists in low- and middle-income countries where field research on gene-drive modified organisms is most likely to occur.

ASSESSING RISKS OF GENE-DRIVE MODIFIED ORGANISMS

The potential for gene drives to spread throughout a population, to persist in the environment, and to cause irreversible effects on organisms and ecosystems calls for a robust method to assess risks. Environmental assessments and environmental impact statements required by the National Environmental Protection Act, though widely acknowledged as valuable in other contexts, are inappropriate tools to characterize the risks of gene-drive modified organisms. Instead, ecological risk assessment would be beneficial in the context of gene drive research, because this method can be used to estimate the probability of immediate and long-term environmental and public health harms *and* benefits.

Ecological risk assessment allows comparisons among alternative strategies, incorporates the concerns of relevant publics, and can be used to identify sources of uncertainty, making it well-suited to inform research directions and support public policy decisions about emerging gene-drive technologies. Two key features of ecological risk assessments are the ability to trace cause-and-effect pathways and the ability to quantify the probability of specific outcomes. This approach could also potentially be built into a structured, adaptive process to oversee the release and management of gene-drive modified organisms in the environment. As of May 2016, no ecological risk assessment has yet been conducted for a gene-drive modified organism.

Some amount of uncertainty is unavoidable. There is currently sufficient knowledge to begin constructing ecological risk assessments for some potential gene-drive modified organisms, including mosquitoes and mice. In some other cases it may be possible to extrapolate from research and risk analyses focused on other genetically-modified organisms and non-indigenous species. However, laboratory studies and confined field tests (or studies that mimic confined field tests such as large cage trials and greenhouse studies) represent the best approaches to reduce uncertainty in an ecological risk assessment, and are likely to be of greatest use to risk assessors.

In the United States, the primary source of federal guidance on ecological risk assessment comes from the U.S. Environmental Protection Agency's 1998 Guidelines for Ecological Risk Assessment. Since 1998 EPA has also published documents update the approaches to specific technical features and incorporate ecosystem services into ecological risk assessment. The 1998 guidelines and subsequent documents focus predominantly on evaluating the risks to ecosystems posed by toxic chemicals, and do not yet adequately address the assessment of multiple stressors and endpoints. Consequently, these documents are not yet sufficient on their own, to guide ecological risk assessment of gene-drive technology. The lack of guidance from the U.S. federal government applicable to ecological risk assessment for the gene drive research community is a critical gap.

PUBLIC ENGAGEMENT

There is broad agreement on the importance of engaging affected communities, stakeholders, and broader publics in decision making about activities involving gene drives. Public engagement can help to frame and define the risks of gene-drive modified organisms and provide input into practical decision making and policy. The outcomes of engagement may be as crucial as the scientific outcomes to decisions about whether to release of a gene-drive modified organism into the environment. Thus, engagement cannot be an afterthought; it requires effort, attention, resources, and advanced planning.

Mechanisms for public engagement and deliberation already exist within some authorized U.S. agencies that oversee biotechnology, but there is generally little clarity on how public engagement should feed into governance and a lack of consensus about best practices in this regard. This is due to at least two factors: first, because regulatory authority remains unclear, the availability of particular formal and customary mechanisms for public engagement also remain unclear; second, although the National Environmental Protection Act will in some cases require public input and afford opportunity for public comment, these mechanisms are an inadequate platform for the more robust forms of engagement discussed in this report.

GOVERNANCE OF GENE DRIVES

The nature of gene drives—which are intended to spread select genetic elements into populations of living organisms—raises many ethical questions and presents a challenge for existing governance paradigms to identify and assess environmental and public health risks. The governance of research begins with the personal responsibility of the investigator, is formalized in professional guidelines, and often extends to legally binding policies and enforceable regulations. In the United States, it is clear that gene drive activities will trigger a variety of governance mechanisms. However, some of these mechanisms may be inadequate for identifying immediate and long-term potential environmental and public health implications of individual gene-drive applications because they lack clarity in their jurisdiction, they are challenged by the distinguishing characteristics of gene drives, or they provide insufficient structures for public engagement.

Two distinguishing characteristics of gene drives, intentional spread a genetic trait through a population and the potential for their effects on ecosystems to be irreversible, present increased uncertainties, making robust assessment of their risk more critical, but also more difficult. Because of the existing uncertainties associated with gene drives, regulation will be needed that facilitates fundamental, applied, and translational research so that the potential harms and benefits of gene drives can be responsibly explored in laboratory and field studies.

It is important to note that a one-size-fits-all approach to governance is not likely to be appropriate. Each phase of research activity—from developing a research plan to post-release surveillance—raises different levels of concern depending on the organism being modified and the type of gene drive being developed. Governance and regulation of gene drive research will need to be proportionate to the hazards posed by the specific activity. In addition, governance will need to be responsive to changes in scientific best practices and ethical considerations as gene-drive technologies develop.

Investigator responsibility and professional guidelines

Currently, institutions, funders, and professional societies work in concert to encourage professional best practices in research. Such cooperation will be instrumental to maintaining high standards in gene drive research. Appropriate resources for education (conceptual) and training (practical) in the responsible conduct of research, as well as public acknowledgement of researchers for their standards of practice, will be important for reinforcing responsible practices in gene drive research.

It is important to note that a one-size-fits-all approach to governance is not likely to be appropriate. Each phase of research activity—from developing a research plan to post-release surveillance—raises different levels of concern depending on the organism being modified and the type of gene drive being developed. Governance and regulation of gene drive research will need to be proportionate to the hazards posed by the specific activity. In addition, governance will need to be responsive to changes in scientific best practices and ethical considerations as gene-drive technologies develop.

Federal guidelines

Laboratory-based research conducted at an institution that receives funding from the National Institutes of Health (NIH) is subject to NIH's guidelines on biosafety and oversight by Institutional Biosafety Committees (IBCs). These guidelines, although international in nature, are adapted to specific institution-

al contexts and are complemented by good laboratory practices. Moreover, the NIH guidelines clearly stipulate that all research at NIH-funded institutions may be regulated by laws established at the local, state, and federal levels, even in the absence of NIH funding for a specific project (e.g., other federal agencies, private foundations). IBCs have provided a robust system of health and environmental protection for laboratory research over the last few decades.

Nonetheless, due to the novel characteristics of gene drives, capacity issues, and an absence of clearly defined guidelines for gene drive research, current IBCs may not have the expertise or resources to evaluate the biosafety of gene drives effectively. IBCs are also not equipped to examine biosecurity or willful misuse issues. However, there is potential to learn from institutional biosafety committees at institutions where gene drive research has been ongoing.

Federal regulations

In the United States, regulation of the gene-drive modified organisms will most likely fall under the Coordinated Framework for the Regulation of Biotechnolgy. However, the US Food and Drug Administration (FDA), the US Department of Agriculture (USDA), and the US Environmental Protection Agency (EPA), the federal agencies included in the current Coordinated Framework, do not have clear lines of authority over the potential applications of gene drive research. The diversity of potential gene-drive modified organisms and contexts in which they might be used reveal a number of regulatory overlaps and gaps. For some potential applications of gene-drive technologies, regulatory jurisdiction may overlap, which suggests the need for a process to quickly determine which agency should coordinate governance of that technology.

Potential dual use issues

Gene drive research raises concerns about biosafety, biosecurity, and potential dual use of the technology. The scientific community, including individual researchers, institutions, and funders, have an obligation to engage in conversations with policy makers about best practices to safeguard against unintentional or intentional misuse of gene-drive modified organisms. Safeguards will be aided by rigorous attention to confinement and containment protocols in laboratory and field tests; active awareness about the potential for misuse; and participation in education and training programs about the dual use potential of gene drive research. Governance mechanisms need to be in place to address questions about the biosecurity implications of gene drive research and consider develop mitigation strategies that are not dependent on the underlying technology.

Need for International coordination

Research on gene drives is global. Responsible governance will need to be international and inclusive, with clearly-defined global regulatory frameworks, policies, and best practice standards for implementation. Low- and middle-income countries where gene-drive modified organisms may be employed will certainly need to be involved in governance, recognizing that many countries lack the capacity to develop a comprehensive regulatory scheme for gene drives from scratch.

The United Nations Convention on Biological Diversity is the main international regulatory instrument governing the development and use of genetically modified organisms, as implemented through the Cartagena and Nagoya Protocols. Many countries are now developing regulatory systems in response to the Cartagena Protocol. Many such systems are predicated on a strong precautionary, nearly preventative approach, which may restrict further gene drive research out of a precautionary concern about gene drives' intrinsic ability to spread and persist in the environment. Given that the United States is not a Party to the Cartagena Protocol, it is a major gap in international governance that the United States does not have a clear policy for collaborating with other countries with divergent systems of governance, especial-

ly when such countries may, in fact, lack the capacity to assess the safety of gene drive research, undertake public engagement and societal dialogue, and maintain regulatory institutions.

In practice, a significant amount of field research on genetically-modified mosquitos operates under guidelines established by international organizations, such as the WHO, and by the research community itself. These should provide a useful foundation for the establishment of guidelines for gene-drive modified organisms. However, these existing guidelines have important gaps and may not address all of the distinctive aspects of gene drives or the range of potential organisms to be used. For example, guidelines may need to be adapted to align to local contexts in order to be implemented. Moreover, most guidelines are not themselves tied explicitly to public oversight and implementation.

GENE DRIVES ON THE HORIZON

There is insufficient evidence available at this time to support the release of gene-drive modified organisms into the environment. However, the potential benefits of gene drives for basic and applied research are significant and justify proceeding with laboratory research and highly-controlled field trials.

A phased-testing pathway and robust ecological risk assessments are essential for navigating uncertainty and informing decisions around the development and application of gene-drive modified organisms.

A comprehensive approach to the development and governance of gene-drive modified organisms will need to go beyond considerations for public health and the environment, and must also consider the benefits of technological innovation, the implications of intellectual property arrangements, public engagement, and economics, among other valued societal commitments.

Specific recommendations related to these overarching conclusions, presented in Chapter 9 of this report, include:

> Recommendation 9-1: Funders of gene drive research should coordinate, and if feasible collaborate, to reduce gaps in knowledge not only about the molecular biology of gene drives, but also in other areas of fundamental and applied research that will be crucial to the responsible development and application of gene drive technology, including population genetics, evolutionary biology, ecosystem dynamics, modeling, ecological risk assessment, and public engagement.
>
> Recommendation 9-2: Funders of gene drive research should establish open access, online repositories of data on gene drives as well as standard operating procedures for gene drive research to share knowledge, improve frameworks for ecological risk assessment, and guide research design and monitoring standards around the world.
>
> Recommendation 9-3: The distinguishing characteristics of gene drives—including their intentional spread and the potential irreversibility of their environmental effects—should be used to frame the societal appraisal of the technology, and they should be considered in ecological risk assessment, public engagement, regulatory reform, and decision making.
>
> Recommendation 9-4*:* Proposed field tests or environmental releases of gene-drive modified organisms should be subject to an ecological risk assessment and structured decision making processes. These processes should include modeling of off-target and non-target effects from the genome level through ecosystem level. When possible, empirical estimates of such variables as gene flow, population change, trophic interactions, and community dynamics should be developed as part of the models.
> Recommendation 9-5: Governing authorities, including research institutions, funders, and regulators, should develop and maintain clear policies and mechanisms for how public engagement will factor into research, ecological risk assessments, and public policy decisions on gene drives. De-

fined mechanisms and avenues for such engagement should be built into the risk assessment and decision-making processes from the beginning.

Recommendation 9-6: In selecting sites for field testing and environmental releases, researchers and funders should be guided by their professional judgement, the feasibility of risk assessment and community engagement, and the community's values and understanding of the balance of benefits and harms. In site selection, preference should be given to locations in countries with the existing scientific capacity and governance frameworks to conduct and oversee the safe investigation of gene drives and development of gene-drive modified organisms.

1

Introduction

Austin Burt had a question: Can an insect's genes be manipulated to stop it from spreading disease? Burt, an evolutionary geneticist, was conducting research on site-specific *selfish genetic elements,* "stretches of DNA" that are certain to pass down from a parent organism to nearly *all* of its offspring (Burt and Trivers, 2006). Like many scientists before him, Burt wondered if the molecular mechanisms that enable selfish genetic elements to spread through a population could be harnessed to eliminate undesirable genetic traits, like the ability of some mosquitoes to carry disease-causing parasites and viruses.

Scientists have known about selfish genetic elements since the late 1880s (Burt and Trivers, 2006). However, the idea to use selfish genetic elements as means to control natural populations did not surface until the mid-20th century. In 1960, George B. Craig, a mosquito biologist, and two of his colleagues, W.A. Hickey and R.C. Vandehey, suggested using a breeding program in which a "male-producing factor" that is naturally present in some male *Aedes aegypti* mosquitoes would be harnessed to control mosquito populations. When male mosquitoes with this male-producing factor breed, most of their offspring then develop as males (Craig et al., 1960). Environmental releases of male mosquitoes carrying this male-producing factor could potentially "reduce the number of females below the [population] level required for efficient disease transmission" (Craig et al., 1960). Hickey and Craig (1966a,b) later described a driving sex determining region on a chromosome in mosquitoes and its potential for population control by causing the sex ratio of the population to shift from half males and half females to an increasing proportion of males. In a related analysis of the conditions favoring the evolution of such biased sex ratios, W. D. Hamilton (1967:479) also realized the potential for using male--bias as a mechanism to control population size. He reasoned that if "the Y chromosome could be freed from the inhibitory control of the rest of the genome," this could be a powerful mechanism of biological control. Chris Curtis, a medical entomologist, then published the first mathematical model demonstrating how a naturally occurring "desirable" gene, such as a gene "to make mosquitoes non-infectible by pathogens," could spread to *fixation* in a population." In the model, the gene would always be present in enough members of the mosquito population to prevent "infectibility" from ever taking hold again (Curtis, 1968).

In the 1960s Craig, Hamilton, Curtis and the other early pioneers did not yet have the molecular tools to engineer "desirable" genes or to molecularly tie them to a biased mechanism of inheritance. More than 30 years of basic biological research in genetics and molecular biology took place before potential genetic engineering tools became available. In 1992, Margaret Kidwell, an evolutionary geneticist, and José Ribeiro, a vector biologist, proposed using *transposable elements*, mobile sequences of DNA, as a mechanism to *drive* an engineered gene into a mosquito population (Kidwell and Ribeiro 1992). In 2003, Burt proposed using *the homing endonuclease gene*, a selfish gene, to drive genetic changes into a natural population (Burt, 2003). A number of geneticists were studying homing endonucleases as a potential basis for targeted gene therapy, a still-experimental approach to treat or prevent particular genetic diseases in humans. Burt extended this reasoning and wondered if homing endonucleases could also be used to drive modified genes through a mosquito population (Burt, 2003; Burt and Trivers, 2006).

Kidwell and Ribeiro (1992) and Burt (2003), in combination with advanced knowledge about genetics and more modern molecular tools, bolstered the field of inquiry into so-called *gene drives*. Geneticists and population biologists continued to explore how to use a variety of selfish genetic elements as the mechanistic basis for the development of a gene drive, primarily in mosquitoes (James, 2005; Rasgon and Gould, 2005; Adelman et al., 2007; Windbichler et al., 2011). However, a precise and predictable mecha-

nism to cause the preferential increase in an existing or engineered trait remained elusive. Then, along came CRISPR (Jinek et al., 2012; Cong et al., 2013).

CRISPR (**C**lustered **r**egularly-**i**nterspaced **s**hort **p**alindromic **r**epeats) are segments of bacterial DNA that, when paired with a specific guide protein, such as Cas9 (**C**RISPR **as**sociated protein 9), can be used to make targeted cuts in an organism's genome. Bacteria use the CRISPR/Cas9 union as a kind of immune system to defend themselves against foreign genetic sequences, such as those that can be inserted by viruses (Barrangou et al., 2007; Hale et al., 2009). Biologists developed a way to use CRISPR/Cas9 like a pair of scissors to make genetic changes by cutting targeted sequences so that existing DNA can be removed or new DNA sequences can be inserted. The CRISPR/Cas9 system, the newest and now most widely used gene editing technique, has rapidly led to breakthroughs in the editing the genomes of many organisms, including plants, worms, flies, fish, monkeys, and human cells (Basset et al., 2013; Friedland et al., 2013; Fu et al., 2013; Jiang et al., 2013; Niu et al., 2014; Gratz et al., 2015). As described by biochemists Sam Sternberg and Jennifer Doudna, "what had once been laborious and time-consuming was now facile and rapidly achievable," because gene editing with CRISPR/Cas9 systems enabled the insertion, deletion, or replacement of specific genes in many species (Sternberg and Doudna, 2015). CRISPR/Cas9 also proved to be a perfect tool to create a gene drive. It enabled biologists to transform the idea of a gene drive into a reality.

In early 2015, three years after the first demonstration of CRISPR/Cas9 as a gene editing tool, a research group led by George Church created the first gene drive in yeast (DiCarlo et al., 2015). Valentino Gantz and Ethan Bier, two molecular biologists, published the first demonstration that a gene drive could be created in an insect, the fruit fly, in March 2015 (Gantz and Bier, 2015). Gantz and Bier used the term *mutagenic chain reaction* to describe the mechanism they developed to create a gene drive using CRISPR/Cas9. By late 2015, two independent research groups, one led by Anthony James and the other by Austin Burt and Andrea Crisanti, developed gene-drive modified mosquitoes (Gantz et al., 2015; Hammond et al., 2016). In less than four years, a new genetic engineering tool, CRISPR/Cas9**,** paired with advanced knowledge about selfish genetic elements, enabled a breakthrough in what scientists had been studying for more than 50 years (see Figure 1-1).

PURPOSE OF THE STUDY

In his seminal paper, Burt posited three reasons for conducting research on gene drives: "to motivate more rapid development of the technology; to warn of containment issues that ought to be addressed during development; and to stimulate discussions on the desirability of eradicating or genetically modifying particular species" (Burt, 2003). Development of the CRISPR/Cas9 technology has accelerated the need to address such issues and more.

Will applications of gene drives be safe? Will they be effective? Will they have unintended consequences for the environment or public health? Do we know enough to release gene-drive modified organisms into the wild? Is using a gene drive to suppress or eliminate a pest species a good idea? What can scientists do to reduce risks to humans, other organisms, and the environment? How do we decide where gene-drive modified organisms might get released? What should governments do? Who gets to decide? These and other questions prompted the National Institutes of Health (NIH) and the Foundation for the National Institutes of Health (FNIH)[1,2] to ask the National Academies of Sciences, Engineering, and Medicine to convene a committee with a broad range of expertise to summarize the scientific discoveries related to gene-drives and considerations for their responsible use. The committee's task includes three primary components: (1) review the state of the science and approaches to reduce unintended harms that

[1]A nonprofit, nongovernmental organization that is separate from the NIH.

[2]This study was sponsored by the National Institutes of Health and the Foundation for the National Institutes of Health, and the National Academy of Sciences Biology Fund. The Defense Advanced Research Projects Agency and The Bill and Melinda Gates Foundation provided support to the NIH and the FNIH, respectively for this study.

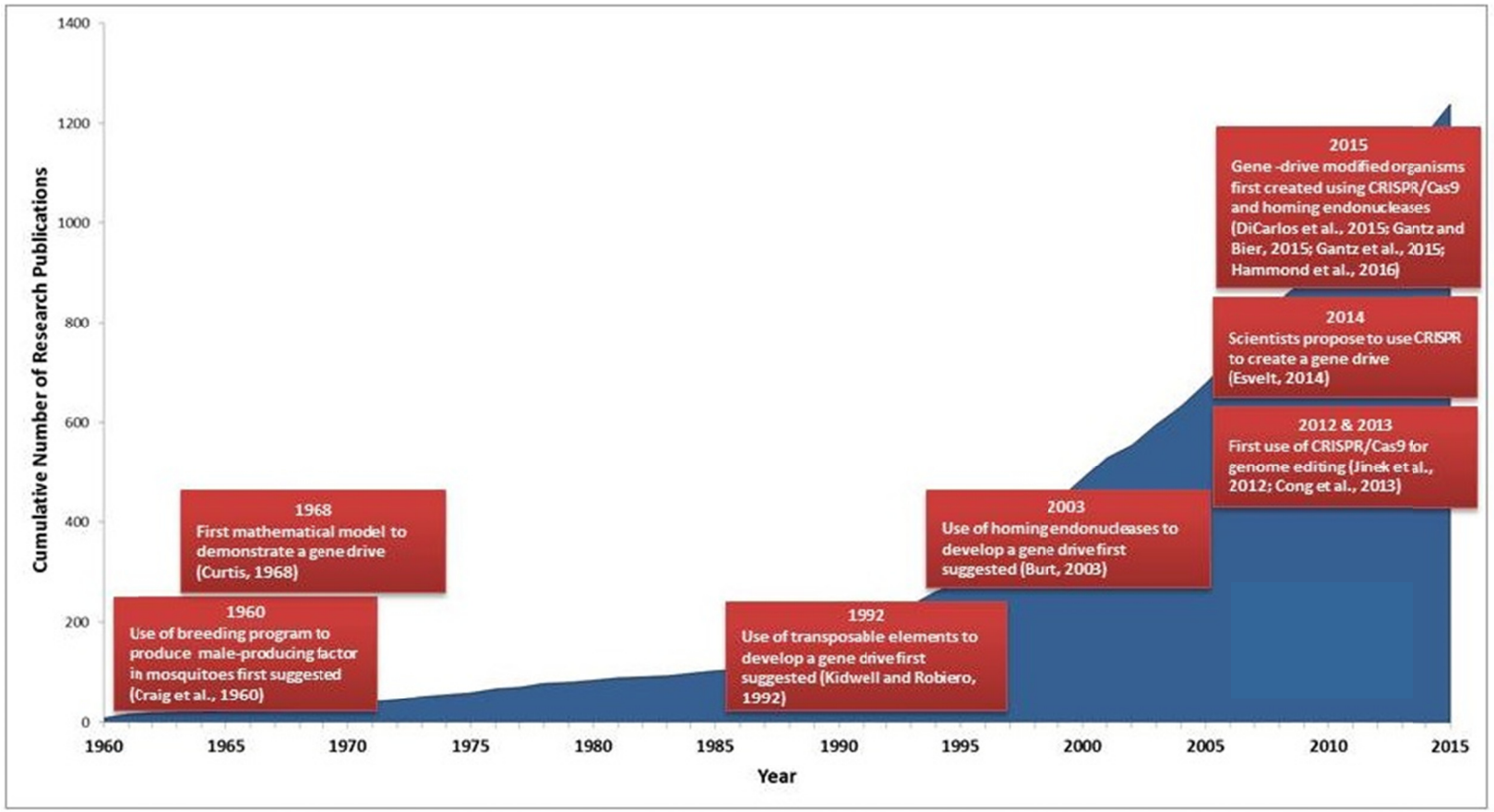


FIGURE 1-1 Cumulative number of gene drive research publications (1960-2015). Boxes highlight selected scientific publications on advances in gene drive research. Data generated via Web of Science (2016) search for peer reviewed articles. Search terms: gene drive, mutagenic chain reaction, meiotic drive, segregation distorter, transmission ratio distortion.

could potentially result from developing and using gene-drive modified organisms; (2) discuss the ethical, legal, and social considerations attendant to field release of gene-drives; and (3) determine the adequacy of existing governance mechanisms and risk assessment guidelines for the environmental and public health implications of using gene drives (see Box 1-1).

To inform this task, the committee held a one-day workshop in Washington, DC, and organized 12 webinars, to gather input from experts and stakeholders. Speakers provided perspectives on science, ethics, public engagement, and governance mechanisms. Topics included the biology of the organisms that are likely initial candidates for gene drive research; evidence derived from experience with field releases of other modified organisms (e.g., use of *Wolbachia*) and how this evidence might inform a risk assessment process for gene drives; how gene drives do, or do not, fit into the current governance system for biotechnology, both in the United States and internationally; how specific values influence public perception of the potential deployment of organisms carrying gene drives; and how best to engage members of the public in discussions about potential benefits and harms of gene drives, particularly in low- and middle- income countries where the first deployments of gene drives to combat vector-borne diseases are likely to occur. The workshop agenda and the list of webinar topics are in Appendices A and B, respectively. The presentations from the workshop and webinars are freely available to members of the public through the project's website.[3] The committee's deliberations led to this final consensus report, which draws on the presentations that the committee heard, the scientific and other literature, and the expertise of its members. General principles to guide responsible practices in gene drive research for the laboratory setting through to field releases are embedded as recommendations throughout the report.

WHAT ARE GENE DRIVES? AND, HOW COULD THEY BE USED?

In reviewing the history of research on what are now called selfish genetic elements, the committee noted differences in the use of terminology and definitions. *Drive*, *gene drive*, *meiotic drive*, *driving Y chromosome*, *selfish gene*, *selfish genetic elements*, and related concepts often have overlapping definitions depending on the historical period and the scientific context in which the terms are used. In this report *gene drives* are defined as systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced. Thus, the result of a gene drive is the preferential increase of a specific *genotype*, the genetic makeup of an organism that determines a specific *phenotype* (trait), from one generation to the next, and potentially throughout the population. These systems encompass the requisite molecular elements *and* events necessary for biased inheritance to occur. Because inheritance is biased in their favor, the genetic elements encompassed by gene drives are often called *selfish genes* or *selfish genetic elements*. Examples of selfish genetic elements include genes or their fragments, all or parts of chromosomes, or noncoding DNA (Burt and Trivers, 2006). As noted above, since the 1960's researchers have imagined that selfish genetic elements "might serve as the basis for 'gene drives' capable of spreading engineered traits through wild populations" (Esvelt et al., 2014).

Gene drives are often described as an exception to the conventional rules of inheritance. First described in 1866 by a monk named Gregor Mendel, the conventional rules of inheritance, also known as *Mendelian inheritance*, dictate that offspring have on average a 50% chance of inheriting a gene from one of their parents. With Mendelian inheritance, not all offspring will inherit the gene, and so the frequency of that gene in future generations will be similar to the frequency of that gene in the parents' generation. With gene drives, offspring have more than a 50% chance of inheriting a genetic element from a parent, and so a specific genotype will increase in the population over time. Figure 1-2 illustrates an idealized difference between Mendelian inheritance and inheritance through a gene drive. However, note that the number of generations and amount of time for a selfish genetic element to spread throughout a population will vary depending on the gene-drive mechanism, the species, and a variety of environmental conditions (see Chapter 2 for additional detail).

[3]http://nas-sites.org/gene-drives

BOX 1-1 Statement of Task

The National Research Council of the National Academy of Sciences will convene an *ad hoc* expert committee in accordance with National Academies' policies to:

- Review the state of the science of gene drive research that relies on genome editing techniques, such as CRISPR/Cas9 and other endonucleases, or other genetic modification approaches. The focus should be on identifying the key scientific techniques for reducing ecological and other risks that should be considered prior to field releases of organisms carrying gene drives. This will require characterizing and assessing environmental and other hazards to target and non-target organisms, and will also include consideration of developing appropriate mitigation strategies, such as reversal drives;
- Using appropriate case studies that are based on likely applications of gene-drive technologies to animals, plants, insect vectors, etc., examine the oversight mechanisms, including guidelines and regulations for:
  - Organisms containing gene drives in the laboratory or other contained, or semi-contained environments;
  - Organisms containing gene drives for use in field releases within the United States; and
  - Organisms containing gene drives for use in field releases in low- and middle-income countries.

This should include examination of the roles of institutional biosafety committee, national or local regulatory authorities, and international frameworks and instruments such as the Cartagena Protocol. An extensive review of international country-specific regulations is not requested, except to the extent that they are illustrative of the general context of oversight or exemplify unique approaches.

- Determine the adequacy of the existing oversight mechanisms and risk assessment guidance to identify the immediate and long term potential environmental and public health implications raised by individual applications of gene-drive technology. This should include safeguarding against accidental or intended misuse spanning the full developmental spectrum from laboratory to release. This may also include identification of gaps that regulators may need to address, although the committee should not attempt to develop specific proposals for new regulations.
- Discuss relevant legal, social, or ethical considerations in selecting sites for field releases and engaging those living in or near potential release sites.
- Provide general principles that will guide responsible practices in gene drive research for the laboratory setting through to field releases for use by investigators, their institutions, the research funders, and regulators.

Gene drives occur in nature through a variety of mechanisms. Researchers are studying how to harness natural mechanisms, such as transposable elements, maternal effect dominant embryonic arrest (Medea), and meiotic drive to develop gene drives in various organisms. However, the pairing of a desired trait with molecular mechanisms that will cause that trait to drive is difficult. CRISPR/Cas9 facilitates the capability to create a gene drive in laboratory populations (DiCarlo et al., 2015; Gantz and Bier, 2015; Gantz et al., 2015; Hammond et al., 2016). In the last few months of 2015 alone, two research studies demonstrated the use of CRISPR/Cas9 to create a gene drive in mosquitoes (Gantz et al., 2015; Hammond et al., 2016.). These studies also demonstrated how a gene drive could be used for two key population control methods:

- *Population suppression*—the spread of a genetic element that causes the number of individuals in a population to decrease; and
- *Population replacement*—the spread of a genetic element through a population that causes a population's genotype to change.

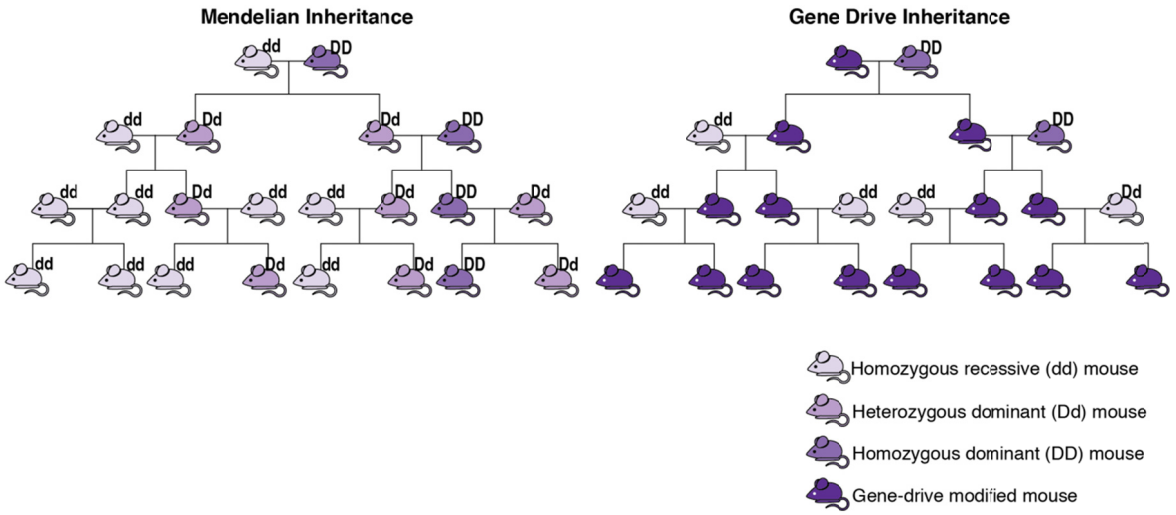


FIGURE 1-2 An idealized illustration of Mendelian inheritance versus gene drive. Under normal Mendelian inheritance (left), offspring have a 50% chance of inheriting a gene. Mating between a mouse homozygous for dominant gene (DD) and a mouse homozygous for recessive gene (dd), produces two heterozygous offspring (Dd). The frequency of the dominant gene (D) does not increase above 50% in any generation of mice. With a gene drive (right), the offspring will almost always receive the targeted genetic element (shown in dark purple), the end result of which is preferential increase of a specific *genotype*. In this idealized illustration, the targeted genetic element is present in 100% of the population. However, note that the number of generations and amount of time for a selfish genetic element to spread throughout a population will vary depending on the drive mechanism, the species, and a variety of environmental conditions.

With the advent of CRISPR/Cas9 as an editing tool, biologists have proposed a wide range of applications for gene drives including to address public health threats, species conservation, agriculture protection, and advance basic research on evolutionary genetics (see Table 1-1). For example, gene drives could potentially be used as one component of an integrated population-control strategy for mosquitoes that transmit malaria, combined with several methods, such as applying insecticides and eliminating breeding habitats. Gene drive technologies could become an important option for addressing complex, difficult-to-solve issues, particularly those where solutions are limited or entirely lacking. For example, outbreaks of mosquito-borne diseases such as Zika virus, chikungunya, West Nile virus, and a diverse group of "neglected tropical diseases,"[4] like elephantitis, can be devastating either because there are few effective treatment options or because affected individuals have limited access to such treatment, particularly in low- and middle-income countries. It is important to recognize, however, that gene drives may be challenging or not possible to develop in many organisms. The enthusiasm for proposed applications must be tempered, as discussed later in this report, by a number of scientific, ethical, legal, and social factors.

Because the field is rapidly advancing, it is reasonable to expect proofs-of-concept in organisms other than fruit flies and mosquitoes within the next few years. The fast moving nature of this field, however, is also a point of concern. For example, it may be easier and much faster to develop a population suppression drive in some organisms than to conduct research on the long term environmental effects. Regulatory systems that govern biosafety and biosecurity are outpaced by the scientific advances, which have led some scientists to propose responsible laboratory practices (Akbari et al., 2015). Gene-drive modified organisms appear to have promise if they can be responsibly developed.

[4]The World Health Organization lists 17 neglected tropic diseases, six of which are transmitted by insect vectors: http://www.who.int/neglected_diseases/diseases/en/.

TABLE 1-1 Potential Applications for Gene Drive Research

Public Health  ***Aedes aegypti*** Image Source: US Centers for Disease Control and Prevention	• Control or alter organisms that carry infectious diseases that affect humans, such as dengue, malaria, Chagas, and Lyme disease • Control or alter organisms that directly cause infection or disease, such as Schistosomiasis • Control or alter organisms that serve as reservoirs of disease, such as bats and rodents
Ecosystem Conservation  ***Hemignathus munroi*** **('Akiapōlā'au honeycreeper)** Image Source: US Department of Fish and Wildlife Service	• Control or alter organisms that carry infectious diseases that threaten the survival of other species • Eliminate invasive species that threaten native ecosystems and biodiversity • Alter organisms that are threatened or endangered.
Agriculture  **Fruit damage from spotted wing drosophila infestation** Image Source: US Department of Agriculture	• Control or alter organisms that damage or carry crop diseases • Eliminate weedy plants that compete with cultivated crops
Basic Research  **DNA Double Helix** Image Source: National Institutes of Health	• Alter model organisms to carry out research on gene-drive function and effects, species biology, and mechanisms of disease

Researchers and the media have expressed concern over the potential social, environmental, and health-related impacts of the development and release of gene-drive modified organisms. Indeed, the prospect of gene drives for the control of vector-borne infectious diseases has sparked significant media interest and concern, particularly in response to the mosquito proof-of-concept publications at the end of 2015 (see Figure 1-3).

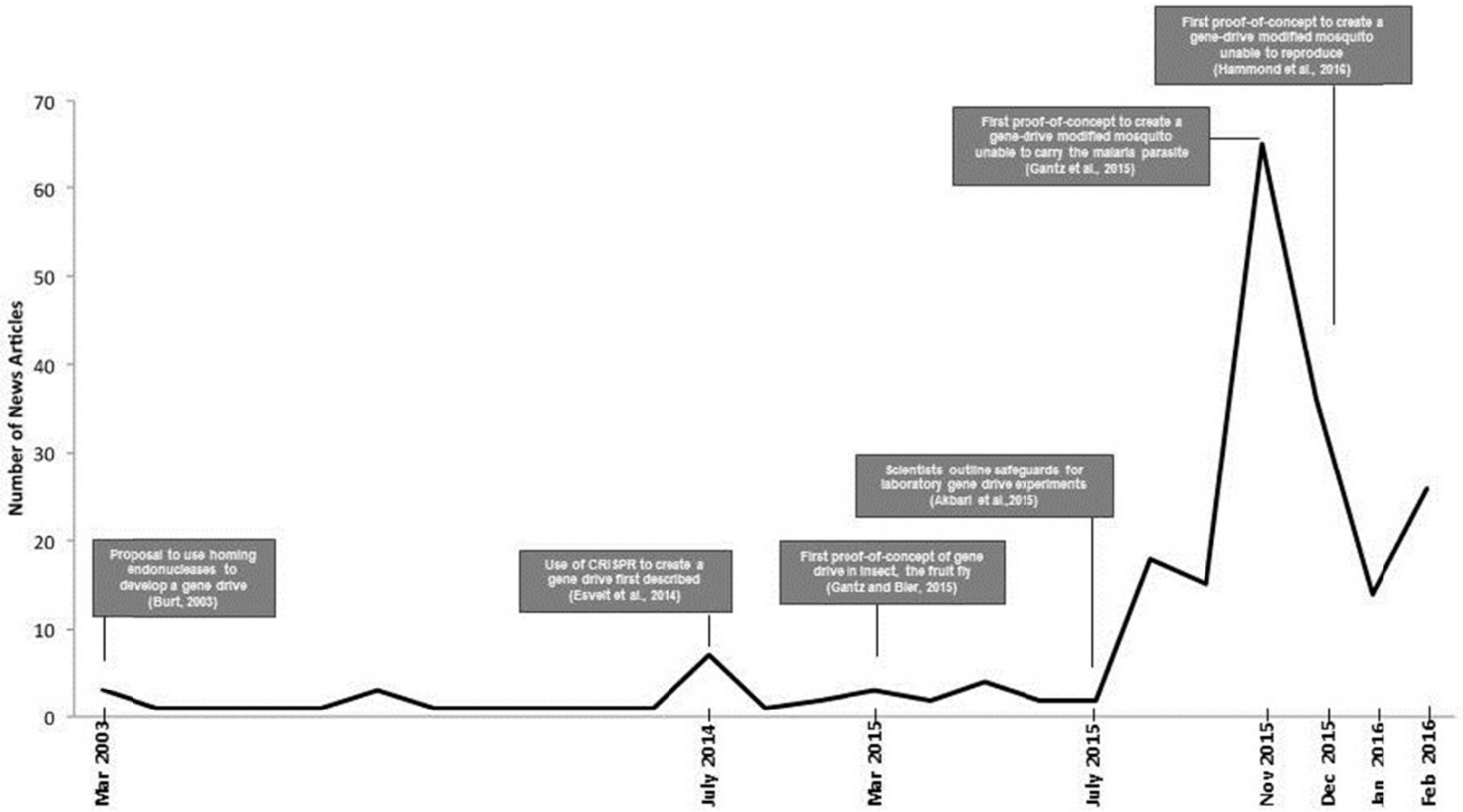


FIGURE 1-3 Timeline of published news articles about gene drive research (2003-2015). Boxes highlight selected scientific publications on advances in gene drive research. Source: LexisNexis, 2016. Limited to top 20 daily circulating newspapers, press releases, and select science publications. Search terms: Gene Drive or Mutagenic Chain Reaction.

Scientific publications cite the benefits of gene drives, but also acknowledge that using gene drives "would represent an entirely new approach to ecological engineering" (Esvelt et al., 2014). Esvelt and colleagues also point out that current knowledge for containing and managing risks related to the spread of novel genes through entire populations and for evaluating ecological consequences is poor. To date, this research has mainly focused on mosquitoes and a few additional organisms for which biological control plans are in place. Similarly, Esvelt and colleagues emphasize that "given the potential for gene drives to alter entire wild populations, and therefore ecosystems, the development of this technology must include robust safeguards and methods of control" (Esvelt et al., 2014). Oye et al. (2014) argued that "studies have evaluated the possibility of releasing transgenic mosquitoes to combat the spread of malaria, dengue, and other mosquito-borne diseases, including requirements for containment, testing, controlled release, and monitoring of mosquito gene drives. This work will need to be replicated and extended for proposed gene drives seeking to alter other species. It is crucial that this rapidly developing technology continue to be evaluated before its use outside the laboratory becomes a reality."

Laboratory research on gene drives is advancing rapidly, but the proposed applications are based on limited proof-of-concept studies. Before this technology is put forth as a safe and viable tool, further research is needed to validate laboratory studies through independent replication of results, assess the potential benefits and harms of gene-drive modified organisms for ecosystems and human health, and develop effective strategies for mitigating potential harmful outcomes. In addition, as the threat of vector-borne diseases and invasive species is not limited by political boundaries, there are many questions about potential governance and regulation of the technology, as well as ethical concerns, and a clear need to engage appropriately with communities in areas where gene drives might be tested and applied in the field.

CASE STUDIES

The committee developed a series of case studies based on the likely directions and applications of gene drive research identified in Table 1-1. The case studies are used to illustrate key considerations for gene drive research, ethics, and governance. See Chapter 3 for the detailed descriptions of the following case studies:

Case Study 1: Using *Aedes aegypti* and *Aedes albopictus* mosquitoes to manage dengue throughout the world

Case Study 2: Using *Anopheles gambiae* mosquitoes to combat human malaria in sub-Saharan Africa

Case Study 3: Using the *Culex quinquefasciatus* mosquitoes to combat avian malaria on the Hawaian islands

Case Study 4: Control populations of non-indigenous *Mus musculus* mice to protect native biodiversity on islands throughout the world

Case Study 5: Controlling non-indigenous *Centaurea maculosa* knapweeds to protect biodiversity in rangelands and forests in the United States

Case Study 6: Controlling *Amaranthus palmeri* (Palmer amaranth, also known as pigweed) to increase agriculture productivity in the Southern United States

Case Study 7: Developing a vertebrate model for gene drive research using *Danio rerio zebrafish*

KEY DEFINITIONS AND CONCEPTS

The committee was tasked to assess the science of gene drives along with the ethical, legal, and social implications of developing this basic biology as applied biotechnology. The committee chose to answer this question by focusing on elements of *responsible science* important to the gene drive field as expressed in six areas of scholarship and practice: gene drive research, values, phased testing, risk assessment, engagement, and governance. In the context of this report, a responsible science approach calls for scientific habits of mind that integrate these six areas of scholarship and practice (see Figure 1-4).

Genome Editing and Gene Drives

Genome editing is a technique that allows researchers to insert, delete, or modify DNA to silence, activiate, or otherwise modify an organism's specific genetic characteristics. There are a number of tools used to edit genomes, including the use of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR. Although they share common techniques, genome editing is not necessarily designed to result in a gene drive.

A *gene drive* is a system of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced. Thus, the result of a gene drive is the preferential increase of a specific *genotype*, the genetic makeup of an organism that determines a specific *phenotype* (trait), from one generation to the next, and potentially throughout the population. Discussions about gene editing and gene drives, from molecular biology to population ecology, are elaborated in Chapter 2.

Values

Values are deeply held, complicated, sometimes evolving beliefs about what kinds of things—in human lives and the world at large—should be fostered, protected, or avoided, and therefore about what people should do and what they should not do. They are critical components of human identity and society. Chapter 4 describes human values that may guide our perceptions, decisions, and actions about gene drive research and its potential applications.

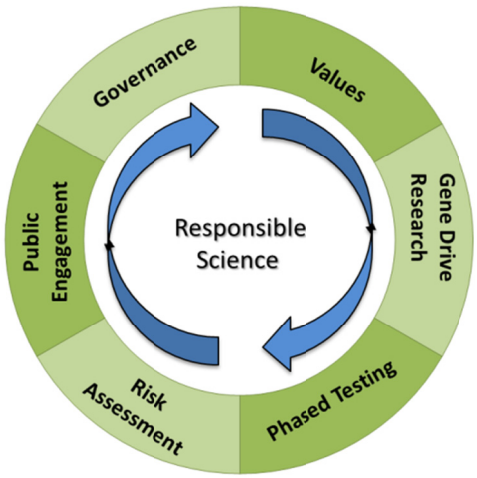


FIGURE 1-4 Responsible science of gene drives as an integration of six areas of scholarship and practice. The committee carefully defined these six areas and described their relevant implications for gene drives research and technologies in individual chapters. Also see the glossary at the end of this report for a comprehensive list of definitions for terms used throughout the report.

Phased Testing Pathway

A phased testing pathway is a step-wise approach to guide the preparation for and conduct of research in the laboratory through environmental release.

The phased testing pathway described in this report is based upon that of the World Health Organization for the testing of genetically modified mosquitoes (WHO, 2014). Chapter 5 describes the phased testing pathway and scientific approaches in each phase to identify and mitigate potential harms of gene drives.

Risk and Risk Assessment

Risk is the probability of an effect on a specific endpoint or set of endpoints due to a specific set of a stressor or stressors. An effect can be beneficial or harmful. For example, a beneficial effect of releasing a gene-drive modified mosquito could be a reduction in the spread of avian malaria, while a harmful effect could be an increase in other types of insects that carry infectious disease.

A risk assessment is the process by which all available evidence on the probability of effects collected, evaluated, and interpreted. Then the potential total effects are estimated from the evidence (EPA, 1984). Risk and risk assessment are discussed in greater detail in Chapter 6.

Public Engagement

Public engagement is the act of seeking and facilitating the sharing and exchange of knowledge, perspectives, and preferences between or among groups who often have differences in expertise, power, and values. Public engagement is a long-term, multidirectional, iterative process of communication. Engagement enables the exchange of information and perspectives as policy questions are asked, refined, reconsidered, and—often only temporarily—answered. Public engagement is discussed in further detail in Chapter 7.

Governance

Governance is the process of exercising oversight through traditions (standards of practice) or regulations by which individuals and communities are held accountable. This includes:

- The process by which authorities are selected, monitored, and replaced;
- The capacity of governing authorities to formulate and implement sound policies; and
- The respect of governed communities for the authorities and processes that govern their economic and social interactions.

Governance in the context of scientific research includes government standard setting and regulation; education of scientists and manufacturers; systems of accreditation; public engagement; and other mechanisms for standards of behavior and controls for safety, environmental protection, and other social goods (NRC, 2015). See Chapter 8 for an in-depth discussion of governance.

CONCLUSIONS

Gene drive research is advancing rapidly, and the proposed applications will likely continue to expand as genome editing tools such as CRISPR become more refined. New scientific information and public perspectives arise almost on a monthly basis concerning the use and application of gene drive research.

The fast-moving nature of this field is both encouraging and a point of concern. Gene-drive modified organisms hold promise for addressing persistent or difficult-to-solve challenges, such as the eradication of vector-borne diseases and the conservation of threatened and endangered species. But the pre-

sumed efficiency of gene-drive modified organisms may lead to calls for their release in perceived crisis situations before there is adequate knowledge of ecological effects, and before mitigation plans for unintended harmful consequences are in place. Continuous evaluation and assessment of the social, environmental, legal, and ethical considerations of gene drives will be needed to develop this technology responsibly and adapt research and governance to the field's complex and emerging challenges.

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2

The State of Knowledge of the Molecular Biology, Population Genetics, and Ecology of Gene-Drive Modified Organisms

For more than 50 years, biologists, geneticists, entomologists, and other scientists have explored approaches to harness gene drives to control or alter natural populations. Scientists have observed gene drives, systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced, in many organisms, including nematodes, plants, rodents (e.g., mice and lemmings), yeast, insects (e.g., fruit flies and mosquitoes) and fish (Boveri, 1887; Dobrovolskaia-Zavadskaia and Kobozieff, 1927; Gershenson, 1928; Rhoades, 1942; Ephrussi et al., 1955; Schultz, 1961; Hickey and Craig, 1966; Bengtsson, 1977; Beeman et al., 1992). Such observations led to proposals to develop gene-drive modified organisms for public health, conservation, agriculture, and other societal purposes, for example, by suppressing populations of mosquito species that transmit human diseases such as malaria, dengue, Zika, and chikungunya among others (Craig et al., 1960; Hamilton, 1967; Esvelt et al., 2014; Campbell et al., 2015).

Two essential components of a gene drive are a silenced (turned-off) or engineered genetic trait (or genetic element that enables the trait to be expressed) and a mechanism to drive the modified genetic element through a population by sexual reproduction. The deployment of cheaper and more user-friendly tools, such as transcription activator-like effector nucleases and CRISPR/Cas9, have facilitated insertion and deletion genetic engineering in many organisms from a single cell to complex multicellular organisms (Sander and Joung, 2014). Such tools, when coupled with driving genetic elements such as transposable elements or homing endonucleases, may enable researchers to mimic naturally occurring gene drive mechanisms. Indeed, recent advances in genome editing techniques using CRISPR/Cas9 as homing endonucleases have enabled researchers to develop gene drives in laboratory populations of yeast, fruit flies, and mosquitoes (DiCarlo et al., 2015; Gantz and Bier, 2015; Gantz et al., 2015; Hammond et al., 2016). The advent of CRISPR/Cas9-enabled gene drives or other gene drive technologies could in principle provide novel approaches to suppress populations or modify the genotypes of populations for pest control, conservation, or other purposes, throughout the world (Esvelt et al., 2014). This chapter has two aims:

- Outline the state of knowledge on genetic elements and their drive mechanisms; and
- Describe primary evolutionary and ecological considerations for the development and potential release of gene-drive modified organisms

The committee discusses the potential for developing gene drives from both molecular and population biology stand points. The discussions include examining how species dispersal can influence the spread of genetic elements through populations, and how ecological impacts can follow from the release of a gene-drive modified organism, particularly one that is designed to reduce or eliminate a population.

SELFISH GENETIC ELEMENTS AND THEIR DRIVE MECHANISMS

As briefly described in Chapter 1, *selfish genetic elements* are sequences of DNA, such as genes or their fragments, all or parts of chromosomes, or noncoding DNA, for which inheritance is biased in their

favor. Selfish genetic elements can "achieve drive" through one or more of three primary mechanisms: *overreplication*, *interference*, and *gonotaxis* (Burt and Trivers, 2006; see Box 2-1). One important particularity of these types of mechanisms is that they do not need to make any contribution to the reproductive success of the host organism in order to drive successfully. *Genes in Conflict* (Burt and Trivers, 2006) provides an in-depth discussion of selfish genetic elements and their drive mechanisms. Here, the committee briefly describes the main types of genetic elements that researchers are using to develop gene-drive modified organisms in the laboratory, and potentially for release into the environment.

Transposable Elements

Transposable elements (TEs), also referred to as transposons or jumping genes, small DNA segments can move from one part of the genome to another by excising themselves and randomly inserting elsewhere in the genome. In the context of a gene drive, transposable elements typify an overreplication mechanism. Multiple copies of the same transposable element often amass in the genome (i.e., increase in copy number) due to DNA repair or gene replication mechanisms that operate in eukaryotic cells. Thus, the copy number of transposable elements typically exceeds what would be expected under Mendelian inheritance.

Plant geneticist Barbara McClintock[1] discovered transposable elements in 1952. She observed that some DNA sequences in maize could occasionally change their location in the genome, and suggested these "controlling elements" could potentially turn genes on and off (McClintock 1951, 1956) Since then, scientists have found that TEs are ubiquitous among eukaryotes and often constitute a major part of the genome (Wicker et al., 2007).

The *P*-element transposon is a well-documented TE in the fruit fly *Drosophila melanogaster*. The *P*-element transposon has long been used to create genetically modified *Drosophila melanogaster* in the laboratory (Rubin and Spradling, 1982). Meister and Grigliatti (1993) first showed that a *P*-element transposon could rapidly spread a specific gene into an experimental *Drosophila melanogaster* population. Although *P*-elements are specific to *Drosophila melanogaster,* the *piggyBac* and *Hermes* TEs have been used for transformation in mosquitoes with varying degrees of success (Fraser, 2012). The use of TEs as vectors for a gene drive has several disadvantages, however, including insertion into random locations, relatively low transforming frequency, limited cargo gene size, and low stability of the integrated sequence (Fraser, 2012).

BOX 2-1 Three Primary Mechanisms to Achieve Drive

Mechanism	Description	Examples
Overreplication	Increased copies of the genetic element within an organism	Transposable elements
Interference	Disrupted replication and transmission of the alternate allele	t-haplotype in mice
Gonotaxis	Biased movement toward the germ line[2]	Abnormal chromosome 10 in maize

Source: Based on Burt and Trivers, 2006, pp 4-8.

[1]Barbara McClintock shared the 1983 Nobel Prize in Physiology or Medicine for her discovery of mobile genetic elements.

[2]A cellular lineage in sexually reproducing organisms that produces the gametes (eggs and sperm) which transmit genetic material to the next generation (Pagel, 2002).

Meiotic Drive

Meiotic drive is an interference gene drive mechanism that refers to genetic alterations that cause a distortion of allelic segregation compared to expected Mendelian inheritance frequencies (McDermott and Noor, 2010).

A well-studied meiotic drive is the Segregation Distorter (SD) autosomal gene complex in *Drosophila melanogaster* (Hiraizumi and Crow, 1960). The SD autosomal gene complex has three elements: an allele of the gene *SD*, an enhancer of segregation distorter E(SD), and a responder (Rsp) locus that is the target of the *SD* gene. The SD interacts with the Rsp in ways still not well understood in order for its effects to be manifest, and, and the E(SD) magnifies these effects (see Larracuente and Presgraves, 2012 for details). When the *SD* autosomal gene complex is present in the male, wild-type[3] *Drosophila melanogaster* sperm do not complete development and only sperm carrying the *SD* autosomal gene complex survive, thus increasing the frequency of the SD complex in the population. Yet, the *SD* autosomal gene complex is present in the *Drosophila melanogaster* population at a relatively low frequency (1-5%) for reasons that are not well understood. Natural meiotic drives have also been found in mosquitoes (Hickey and Craig, 1966; Sweeny and Barr, 1978). In this case, the meiotic drive gene is linked to the male-determining locus (M), which is on an autosome, and the responder gene to the female-determining locus (m) is on the homologous chromosome. The meiotic drive product causes the breakage of the female-determining autosome. When the allele is present in the male, no females are produced, leading to a highly biased sex ratio in favor of males as long as the local population has no resistance alleles.

In vertebrates, the most studied natural meiotic drive is the *t*-haplotype in the house mouse *Mus musculus* (Silver, 1993; Ardlie, 1998). The *t*-haplotype consists of a series of linked, independent *T* complex distorter genes and a *T* complex responder gene that are inherited together. When present in the heterozygous (*Tt*) condition in the male, the wild-type sperm show motility defects and are functionally inactive, so more than 90% of the progeny receive the *t*-haplotype. The sterility of the *Tt* males, the presence of recessive lethal mutations within the *t*-haplotype, and a number of non-genetic factors, such as multiple matings and population size, serve to maintain the *t*-haplotype at a low frequency in a population (Ardlie, 1998).

Meiotic drive also occurs in plants. For example, the Abnormal 10 (Ab10) chromosome of maize (*Zea mays* ssp. *mays*) is a modified version of chromosome 10 linked to factors that cause segregation distortion (Rhoades and Dempsey, 1985). Ab10 affects the segregation of chromosome 10 and also affects unlinked chromosomes if they contain chromosomal knobs (small heterochromatic regions that sometimes act as neocentromeres during meiosis to allow chromosomes to be pulled apart). In the presence of Ab10, a knobbed chromosome of a heterozygous chromosomal pair segregates into about 70%, instead of the expected 50%, of viable megaspores (Rhoades, 1942). In theory, the Ab10 system can drive itself to fixation while simultaneously causing unlinked maize chromosomes to have ever-increasing chromosomal knobs. However, the Ab10 chromosome tends to be rare in natural populations, perhaps because its spread is constrained by the size and architecture of chromosomes during segregation (Buckler et al., 1999). Additional segregation distorters have been identified in other plant species, such as skewed sex ratios in *Silene* (Correns, 1906; Delph and Carrol, 2001) and skewed chromosomal segregation in monkeyflower hybrids (Fishman and Saunders, 2008). Generally, the formation of neocentromeres in plants and other organisms often appears to be a product of meiotic drive (Dawe and Hiatt, 2004), perhaps reflecting rapidly changing interactions among centromeric components (Henikoff et al., 2001).

Underdominance

Underdominance, or heterozygous disadvantage, occurs when the heterozygous progeny "have a lower relative fitness than both [parental] homozygotes" (Altrock et al., 2011). Curtis (1968) proposed

[3]The collection of genotypes or alleles found in a natural populations. Natural populations harbor substantial amounts of genetic variation, so there is rarely a single wild-type genotype or allele.

that fertile chromosomal translocation homozygotes could be used to drive a gene into a pest population since the heterozygote is semi-sterile (as evidenced by the fact it produces about 50% of the expected progeny). Researchers attempted this approach but met with little success for various technical reasons (Curtis, 1985; Sinkins and Gould, 2006). In the past 15 years several models for using engineered underdominance for pest control were proposed, including those of Davis et al. (2001), Magori and Gould (2006), and Altrock et al. (2010). One approach that has been tested in laboratory populations is the maternal-effect lethal underdominance system (UD^{MEL}) in *Drosophila melanogaster* (Akbari et al., 2013). The UD^{MEL} system includes two maternal toxins targeting maternal genes essential for embryonic development and two antidotes (Akbari et al., 2013). The maternal toxin A is linked to the antidote B, and maternal toxin B is linked to the antidote A. The two constructs can be situated at the same position on homologous chromosomes or on different chromosomes, and the offspring must receive both constructs to survive. This requirement will only be met if the number of transgenic organisms released is above a certain threshold; otherwise, the transgenes will be lost from the population. In *Drosophila,* this method has been used both to drive a transgene to fixation through males carrying the transgenes and to remove a transgene from the population by increasing the ratio of wild-type males and females relative to the ratio of transgenic flies (Akbari et al., 2013). Similar methods have been proposed and modeled but not tested in the laboratory, including *Semele* (Marshall et al., 2011) and *Medusa* (Marshall and Hay, 2014). *Semele* is a toxin-antidote system in which a semen-specific toxin is carried in transgenic males and an antidote is carried in transgenic females. Wild-type females that meet with the transgenic males are either killed or unable to produce offspring, which leads to population suppression (Marshall et al., 2011). When both transgenic males and females are released, the transgenes and any cargo gene that they contain will become fixed in the population. In the *Medusa* system, maternal toxin A and zygotic antidote B are on the X-chromosome whereas zygotic toxin B and zygotic antidote A are on the Y chromosome. At least two releases of males bearing both transgenic chromosomes are needed for suppression of the female population. Both of these methods require a high release threshold to be driven into the population.

Other approaches for establishing underdominance are also being tested. For example, Reeves et al. (2014) are using an RNA interference (RNAi) approach in *Drosophila melanogaster* to suppress an endogenous gene that is haploinsufficient (that is, the gene must be present in two copies for normal development) coupled with an RNAi-insensitive rescue version of the gene.

Maternal-Effect Dominant Embryonic Arrest

Maternal-effect dominant embryonic arrest (*Medea*) is a natural genetic element that was first discovered in the flour beetle (*Tribolium castaneum*) and causes maternal-effect lethality in all offspring that lack the Medea-bearing chromosome (Beeman et al., 1992). Synthetic *Medea* elements, consisting of a microRNA that targets and silences a maternal gene necessary for embryonic development (maternal toxin) linked to a zygotic antidote gene that rescues that function, have been inserted in the *Drosophila melanogaster* genome using the P-element transposon (Chen et al., 2007; Akbari et al., 2014). In these instances the chromosome carrying the *Medea* element replaced the wild-type chromosome in about 16 generations. This element can carry a cargo gene into the population and potentially can be used for population suppression (Akbari et al., 2014).

Homing Endonuclease Genes

Homing endonuclease genes (HEGs) are situated on a chromosome within a specific sequence that they recognize and cut. These genes encode enzymes that work by cutting the recognition sequence on the chromosome that is homologous to the one originally containing the HEG. After the sequence is cut, homologous recombination is used to then copy the HEG into the cut homologous chromosome. When "this process occurs in the germline, the proportion of gametes that contain the HEG is greater than 50%" (Fraser, 2012), and therefore the HEG could theoretically drive itself through the population. HEGs are pre-

sent in eukaryotic organisms, archaea, and bacteria, where their recognition sequences are found at low frequencies in the genome (Jasin, 1996).

Austin Burt (2003) first proposed the idea of using HEGs to develop a gene drive. Windbichler et al. (2011) later described the use of an HEG in the creation of a gene drive in mosquitoes. In this instance, a transgenic mosquito was created with a cleavage site near a fluorescence gene, and, upon expression of the HEG from a donor DNA plasmid, the site was cut, allowing for copying of the HEG into the target site through gene repair and homologous recombination. One limitation of this system is that the DNA recognition and cleavage functions of these HEGs are very much intertwined (Sander and Joung, 2014). Furthermore, this method requires the ability to easily generate an HEG cleavage site in the target gene of interest, limiting the use of HEGs for editing purposes (unless the site is found naturally in the target gene). Building on the concept of meiotic drive described earlier, Galizi et al. (2014) used a specific HEG called the "X-shredder" to distort artificially the sex ratio in *Anopheles gambiae* by targeting a specific sequence on the X chromosome for disruption. This "X-shredder" mechanism, in turn, led to the loss of females (population suppression) and the subsequent bias towards male progeny. This mechanism, however, can only work if the sequence of interest is found on the X chromosome and is (ideally) repetitive in nature, due to the mechanism of repair employed by the cell.

In addition, the *T* complex distorter genes are now being considered as a means of introducing the sex-determining *Sry* gene into genetic (XX) females so they develop as males but are sterile (Campbell et al., 2015). Case study 4 of this report (see Chapter 3) summarizes the use of this type of gene drive to eradicate invasive rodents on islands.

Zinc Finger Nucleases

Zinc finger nucleases (ZFNs), an alternative to HEGs, are engineered DNA binding proteins that facilitate targeted editing of the genome (Pratt et al., 2012; Figure 2-1). ZFNs combine a nuclease domain derived from a specific restriction enzyme (typically *Fok*I) with a DNA binding domain mediated by zinc fingers and can be used to target user-defined DNA sequences (Kim et al., 1996). The ZFNs function as pairs because the enzymatic domains must form dimers in order to cleave DNA (Urnov et al., 2010). However, ZFNs can cleave other sequences besides the intended one, are sometimes toxic to cells (Cornu et al., 2008), and must be custom-made, making them a more expensive method for editing (Koo et al., 2015).

Transcription Activator-Like Effector Nucleases

Like ZFNs, Transcription Activator-Like Effector Nucleases (TALENs) utilize the same nuclease domain and function as dimers but instead rely on a DNA binding domain called a TAL effector derived from the plant pathogenic bacterium *Xanthomonas* (Boch and Bonas, 2010; see Figure 2-1). These TAL effector binding sites recognize single bases such that four different sites (unique to each of the four bases that constitute DNA) can be generated (Boch et al., 2009). Their creation can be quite time-consuming and labor-intensive, as TALENs require a new protein pair to be created for every DNA sequence to be edited. Choosing sequences that differ by at least seven base pairs from I other sites and using software to generate site-specific TALENs[4] has been helpful in creating functional TALENs (reviewed in Koo et al., 2015). Simoni et al. (2014) showed that ZFNs and TALENs could be used as gene drives, with homing frequencies of 34% and 49% to available target loci, respectively, in *Drosophila melanogaster*. In many instances, though, TALENs are not transmitted faithfully due to the number of repetitive elements required and their subsequent tendency to recombine, leading to their loss of function (reviewed in Koo et al., 2015).

[4]www.talenlibrary.net.

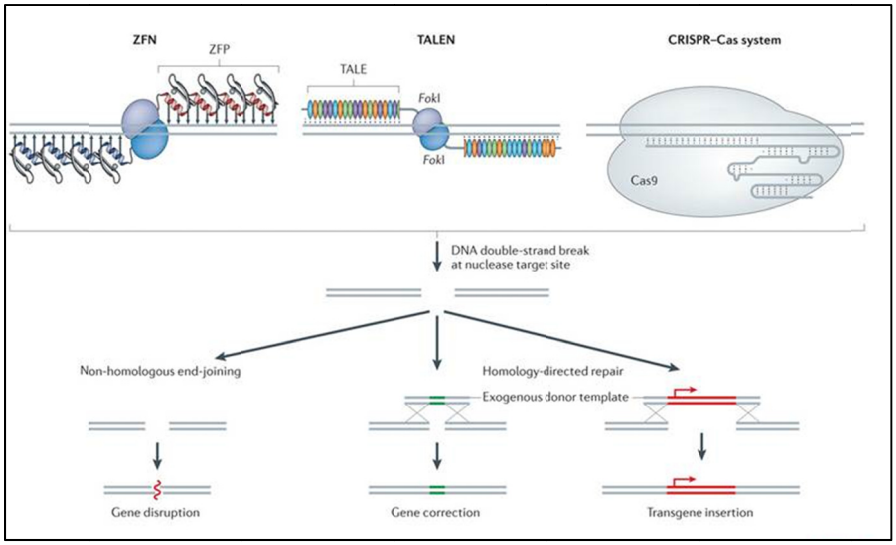


FIGURE 2-1 Illustration of three gene editing techniques. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein systems can introduce double strand breaks DNA at specific locations using different molecular mechanisms. Source: Adapted from Yin et al., 2014.

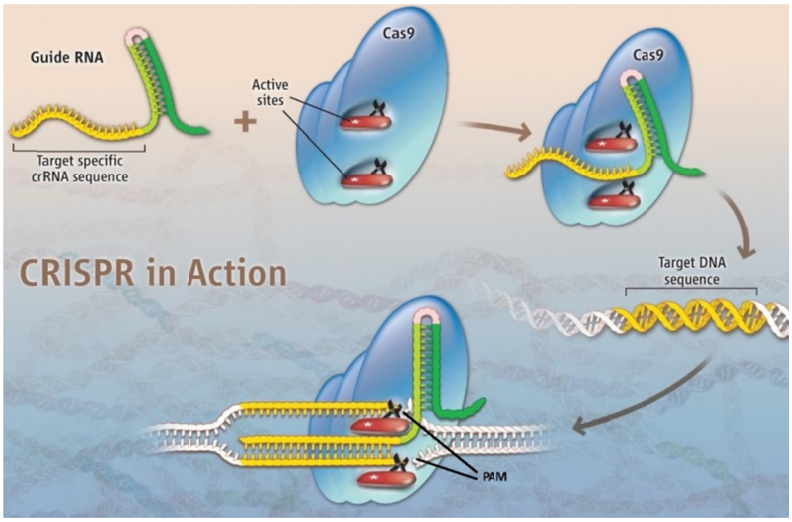


FIGURE 2-2 The CRISPR/Cas9 System in Action. Through complementary base pairing between the guide RNA and the genomic DNA target, the guide RNA directs Cas9 to the DNA, leading to a blunt-ended, double-stranded break within the target that is close to a specific sequence called the protospacer adjacent motif, which is the sequence recognized by Cas9. Source: Adapted from Pennisi, 2013.

CRISPR/CAS9-BASED GENE DRIVES

CRISPR/Cas is a genetic engineering tool developed from an adaptive immune system like response observed in bacteria and archaea. The CRISPR/Cas9 system requires a target-specific guide RNA (gRNA) and a CRISPR associated protein (Cas9), which is an enzyme that cleaves DNA (Jinek et al., 2012; Bolukbasi et al., 2015; Sternberg and Doudna, 2015; see Figures 2-1 and 2-2).

Compared to ZFNs and TALENs, the CRISPR/Cas system is a less expensive and less laborious method for genetic engineering, and also can be effectively used to target multiple genes at once through the introduction of relevant gRNAs (Bono et al., 2015).

Scientists have used the CRISPR/Cas9 system to developed a gene drive in the laboratory in several organisms, including fruit flies, mosquitoes, and yeast (DiCarlo et al., 2015; Gantz and Bier, 2015; Gantz et al., 2015; Hammond et al., 2016). The CRISPR/Cas9 system can insert a particular gene into a chromosome, resulting in one copy of the gene drive in the genome. The inserted gene drive then "cuts" the wild-type homologous chromosome. Using the inserted gene drive as the template, the DNA repair machinery inserts a copy of the gene drive into the wild-type homologous chromosome, resulting in two copies of the gene drive in the genome (Sander and Joung, 2014). Thus, all of the gene-drive modified organism's offspring will inherit one copy of the gene drive (see Box 2-2). The CRISPR/Cas9 system therefore increases the likelihood that an organism will pass on a particular gene, and could be used for engineering a gene-drive modified organism to drive a gene through a population (Webber et al., 2015).

In 2015, researchers published four proof-of-concept studies demonstrating the use of CRISPR/Cas9 to develop gene drives in the yeast *Saccharomyces cerevisiae* (DiCarlo et al., 2015), the fruit fly *Drosophila melanogaster* (Gantz and Bier, 2015) and two mosquito species, *Anopheles stephensi* (Gantz et al., 2015) and *Anopheles gambiae* (Hammond et al., 2016).

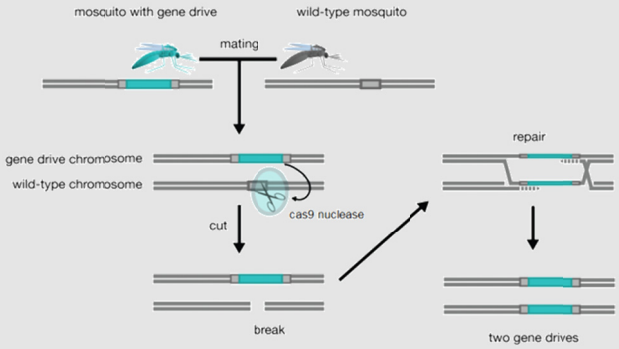
For their development of a gene drive in yeast, DiCarlo et al. (2015) used a split-gene drive[5] in which Cas9 and the guide RNA used for targeting were physically separated. Only when Cas9 was present was the targeted gene disrupted. Using this technique, the researchers achieved a highly efficient disruption that is capable of carrying a cargo gene into the site with the same high efficiency. The drive also was highly efficient in various genetic backgrounds. Their experiments also showed that the edited gene sequence could be restored with an overwriting drive that contained an intact copy of the gene although the Cas9 and guide RNA remained in the genome. In addition, DiCarlo et al. (2015) showed that using a much larger construct containing both Cas9 and the guide RNA was also highly efficient. The purpose of this research was to find safer ways to develop gene drives in various organisms.

In the fruit fly, Drosophila melanogaster, Gantz and Bier (2015) created a gene drive construct containing Cas9 under the control of DNA sequences (promoters) that would cause its expression in both germline and somatic cells linked to a guide RNA. The guide RNA targeted a particular site in the *yellow* body color gene of *Drosophila*. Injection of this construct into wild-type embryos yielded flies that, when mated to wild-type fruit flies, produced yellow (y- y-**)** female progeny rather than the expected females with a darker body color (y+ y-**),** showing that the gene on both chromosomes had been disrupted. When these y- y- females were mated to wild-type males, 97% of the female progeny were y- y-, indicating that the insertion was transmitted for at least two generations. However, phenotypic mosaicism was found in some of these females.

Gantz et al. (2015) used the same basic strategy as Gantz and Bier (2015) to drive two anti- parasite genes along with a fluorescent eye color marker into *Anopheles stephensi*, a mosquito vector of the malaria parasite. They found a 98.8% gene conversion rate in the third generation of both gene-drive modified males and females mated with wild-type mosquitoes, and that the anti-parasite genes were transcriptionally active. However, they noted maternal effects due to activity of Cas9 in the embryo so that inheritance of the gene drives were decreased resulting in near-Mendelian ratios of the progeny. There were also fewer progeny, indicating that the chosen insertion site (in the eye color gene) may not be the optimal site for use in making transgenic mosquitoes for release. Gantz et al. (2015) concluded that the gene drive should be restricted to the germ line and that additional work is needed to find the best site for insertion and the most efficacious anti-parasite genes to use.

[5]When gene drive components (Cas9, gRNA, and donor template) are supplied separately to the organism. See Chapter 5 for additional details on this approach.

BOX 2-2 A Conceptual Recipe for a CRISPR/Cas9-Based Gene Drive



The CRISPR/Cas9 gene editing system can be used to increase the likelihood that a gene will be inherited by an organism's offspring. The conceptual recipe to develop the gene drive includes five steps:

1. Select a target site to be disrupted within the organism's genome.
2. Select the gene that will be driven through the population.
3. Engineer the driving construct needed for insertion of the gene into the target site.
4. Ensure that mechanisms are in place to copy the inserted gene onto the homologous chromosome during DNA repair.
5. Validate that the copy mechanisms result in insertion of the gene into the homologous chromosome.

Source: Figure adapted from Esvelt et al., 2014.

In their research on the mosquito *Anopheles gambiae,* Hammond et al. (2016) used a CRISPR/Cas9-based gene drive to disrupt three different putative female-fertility genes. The construct was similar to that of Gantz et al. (2015) except that it lacked the anti-parasite genes. For all three genes, the inserted gene drive construct efficiently copied itself into the gene on the homologous chromosome causing sterility of female homozygotes, but also severely decreased the fertility of the heterozygous females. Modeling showed that this reduced reproductive capability of the heterozygotes would lead to the disappearance of the gene drive from the population over time for two of the three inserted genes. Population cage studies with the gene that showed the highest insertion efficiency and higher heterozygous female fertility revealed an increase in the frequency of the insertion from 50% to 75% over four generations.

In addition to the need to refine methods to develop CRISPR/Cas9-based gene drives in yeast, fruit flies, and mosquitoes, another important consideration is whether these scientific findings will be applicable to other organisms. For example, could a CRISPR/Cas9-based gene drive be developed in vertebrate animals (e.g., fish and rodents), or for use in plants?

Researchers have made considerable progress in understanding the genome and how it might be manipulated using a gene drive. However, such research is in an early stage. While high quality laboratory work demonstrates the application of gene drives in the laboratory, additional research is needed to refine gene drive technology and understand its effects before gene-drive modified organisms can be release in the environment.

POPULATION ECOLOGY AND ECOSYSTEM CONSIDERATIONS

Although molecular biology research on gene drives is rapidly advancing, extensive research on population dynamics, evolutionary processes, and ecology of gene-drive modified organisms has not yet taken place. Releasing gene drives into the environment means that complex molecular systems will be

introduced into complex ecological systems, setting off a cascade of eco-evolutionary dynamics. Key considerations include fitness, species dispersal, gene flow, ecosystem dynamics, and evolution. Changes in population dynamics will influence evolutionary processes and vice versa. Advances in theory, modeling, and empirical studies will be needed to understand and better understand the effect of gene-drive modified organisms on these complex processes.

The Role of Evolutionary Fitness

The success or failure of a gene-drive modified organism will depend on the evolutionary fitness of the organism. Fitness is, most simply, the number of offspring that an individual contributes to the next generation. When discussing the fitness of individuals with different genotypes (in the context of this report, the individuals that do and do not carry a gene drive) is the average number of offspring contributed by each genotype, which tells us how many of each type of gene (the gene drive or its alternative, wild-type form) will populate the next generation. The average fitness of a genotype is measured by combining the rate at which different genotypes survive to reproduce with the number of offspring of contributed by those that do survive to reproduce (Orr, 2009).

The fitness of an individual organism may be measured *absolutely*, as the total number of surviving offspring that it produces during its lifetime, or relatively, as a proportion of the highest value absolute fitness seen in another individual. Relative fitness is the usual standard for comparing genotypes; a genotype whose carriers leave only 80% as many offspring, on average, as those left by the genotype with the highest absolute fitness is said to have a relative fitness of 0.80. A variety of empirical methods have been used to estimate the relative fitness of a particular genotype compared to other genotypes in a population, especially by tracking their comparative ability to produce offspring in future generations (Prout, 1965; Burt, 1995; Mueller, 2009).

A final important quantity is the *mean fitness* of a population. When describing absolute fitness, the mean fitness is, approximately, the ecological replacement rate: How many offspring are, on average, left behind by one individual? If the mean absolute fitness is greater than 1.0, the population will grow in size in the next generation and if the mean absolute fitness is less than 1.0, the population will decrease in size. It is important to distinguish relative and absolute fitness in gene-drive applications because measures of relative fitness may not reveal how a gene drive will affect the actual numbers of individuals. When population suppression is the goal of deploying a gene drive, it is essential to understanding mean fitness in absolute terms.

The fitness of an individual can be affected by small genetic changes, such as the introduction of a point mutation or a gene drive. Introduced mutations may have a positive effect on fitness or a negligible effect, but more often they are expected to decrease the fitness of their carriers. However, the magnitude and direction of the fitness effect caused by a mutation at one gene or the insertion of a gene drive at one location can also depend on the other genes carried by that individual. This is because interactions between the mutation and other loci in the genome can affect the phenotype of the organism and its fitness (de Visser and Krug, 2014). Evidence of such interactions can be found when the fitness of a mutation or genetic modification varies among genetic stocks or lines derived from a target population (e.g., Amenya et al., 2010). These interactions are known as epistatic effects. Thus, a rigorous examination of the fitness consequences of introduced genetic material requires measurement of its effects across multiple genetic backgrounds. For this reason, it is sometimes useful to measure the mean fitness of a population with a mutation or gene drive because that mean will be based on the total collection of genotypes in the population. If one or more new genotypes are introduced into a population, mean fitness may increase, decrease, or remain the same.

The measure of fitness effects is relevant to gene drive applications because it is the basis for estimating the rate of spread of the gene drive through a population. The conceptual foundation for these estimates comes from the population genetics literature, particularly the models of natural meiotic drives developed by Hartl (1970), who in turn built on previous models for the t-allele system in mice (Lewontin, 1968). In these models, the fitness of an organism that contains the gene drive is one key pa-

rameter, but there is another important parameter: the rate at which the drive allele converts the other, non-drive allele in a heterozygous individual. For example, when a heterozygous individual always produces gametes with only the drive allele, the conversion rate is 100%.

These population genetic models illustrate that the basic dynamics of gene drives are propelled by the conversion rate of the gene drive and the fitness of individuals that have the drive. When the drive has no effect on fitness and only acts through the conversion process, the gene drive spreads rapidly through a population until all individuals are homozygous for the drive. This can happen in as few as a dozen generations, assuming that enough gene drive individuals are released initially to drive the process deterministically (Unckless et al., 2015). The rate of spread of the gene drive is even faster if the drive is beneficial—that is, if the gene drive increases the fitness of its carriers. Speed of gene drive spread is also strongly influenced by generation time; the shorter the generation time of a species, the faster the spread.

When the gene drive decreases the fitness of the organism (that is, when it carries a cost), the results depend on the balance between the conversion rate (which increases the frequency of the drive) and the cost of the drive (which decreases its frequency) (Burt, 2003). In the simplest case, when the drive is lethal in the homozygous condition but has no effect on the fitness of heterozygote carriers, the drive reaches an equilibrium frequency equal to its conversion rate. When the conversion rate is very low and the fitness cost to homozygotes carrying the drive is very high, this equilibrium frequency is itself very low, and the drive will not spread through the population and might even be lost. When the gene drive affects the fitness of heterozygote carriers as well as the fitness of homozygote carriers, the cost to the heterozygous individuals can determine whether that equilibrium is stable or unstable (Deredec et al., 2008; Unckless et al., 2015). If it is unstable, then introductions of the drive must be done at frequencies that exceed that equilibrium value if the drive is to spread, a situation not unlike the population genetics of control systems using *Wolbachia* strains (Turelli and Hoffmann, 1999).

These models show an important characteristic of a gene drive; namely, it can spread throughout a population even if it reduces the fitness of individuals that carry it. This is an especially important property when the goal of deploying a gene drive is population suppression (e.g., reducing the population density of a disease vector). In many cases, the goal of deploying a gene drive will be to modify the genetic constitution of a population, for example, to prevent a disease vector from acquiring or transmitting a pathogen. For either goal, the approach requires that the altered genotype can survive in the environment and contribute to sexual reproduction; otherwise the introduced gene cannot spread into the target population. If suppression is the goal, the fitness effect of the introduced gene may be as extreme as lethality (fitness of zero), and preliminary experiments can be conducted to confirm that this effect occurs regardless of the genetic background of individuals that inherit the gene in the target population. If modification is the goal, the fitness effect of the introduced gene must be non-lethal, because replacement of individuals in the target population is the desired outcome. However, even in the case of modification, low fitness of the engineered genotype and those inheriting the gene may be desirable in order to facilitate creating a "self-limiting" gene drive that would either be very restricted in its spatial dissemination or lost after a certain number of generations (Gould et al., 2008; Legros et al., 2013).

Species Dispersal and Gene Flow Among Populations

The models of Hartl (1970) and others (Deredec et al., 2008; Unckless et al., 2015) are important for generating expectations about the spread of gene drives through a population, and similar models will be useful for risk assessment. However, like most population models, these contain simplifying assumptions for mathematical tractability, such as the assumption that there is only one population of constant size. In reality, populations are often spatially structured with some genetic migration among them.

Understanding the patterns of a species' spatial structure and how genes move among populations are important components to understand when preparing to release a gene-drive modified organism. Researchers can develop prospective simulations that model the target species and help estimate the number of gene-drive modified individuals to introduce or guide the spatial distribution of introductions. However, data on movement patterns and their effects on spatial structure may not always be available. Thus,

models can also be informed by what is known about spatial structure for a variety of other organisms. The following sections focus on some of the properties of gene dispersal and its potential effects—both beneficial and detrimental—that can inform the application of gene drives and aid in planning their release.

Types of dispersal among populations

The promise of gene drives is based on the potential spread of the desired gene through an entire area occupied by a species or population. The spread itself occurs via the movement of individuals or gametes from one location to another, with subsequent mating and reproduction. The spread of genes via movement between populations is called gene flow (Slatkin, 1987). Understanding the role of gene flow is critical for determining how rapidly a gene drive will spread among populations, whether the goal is to move the drive into additional populations or, conversely to limit its spread. Understanding gene flow is also vital for estimating the likelihood that the gene drive may move into a non-target population.

The diversity of gene flow patterns are influenced by three main factors: the stage of the life cycle in which the movement of individual organisms among populations is most likely, the type of movement through which individuals carry genes among populations, and the spatial scale over which movement typically occurs.

Gene flow may occur by the movement of either whole organisms or gametes. For many species, "typical" movement of an individual occurs in specific life cycle stages. For example, in many organisms, movement occurs via dispersal of fertilized eggs (especially in marine animals, e.g., D'Aloia et al., 2015), seeds (as in vascular plants, e.g., Picard et al., 2015; Shao et al., 2015), or spores (as in fungi, ferns, and mosses, for example.). By contrast, in many animals, movement among populations is most likely when juveniles or young adults of one gender disperse from the area of their birth to establish themselves elsewhere (Graw et al., 2016). In these cases, social interactions can play a critical role in determining individual movement, where an individual settles, and whether movement results in breeding and actual gene flow (Booth et al., 2009, Wey et al. 2016). The stage of the life cycle in which gene flow occurs can influence the rate at which genes move from one population into another. For example, the passive dispersal of fertilized eggs and seeds can introduce substantial numbers of genes from one population into another (Ceron-Souza et al., 2015), whereas the dispersal of juvenile or adult individuals in search of new habitat will generate much lower rates of gene exchange (Craig et al., 2015).

In contrast, many plants and some marine invertebrates disperse primarily through the movement of gametes rather than whole organisms. The most familiar example is wind-borne pollen, which can transport genes across long distances (Huang et al., 2015). In many cases, especially when pollen movement is facilitated by insect pollinators, the movement of genes can be quite circumscribed (Tambarussi et al., 2015). Gene flow via gametes is fundamentally different from gene flow via movement of individual organisms in two ways. First, it represents sexual transfer of a haploid genome rather than the movement of a diploid genome. Second, it offers a greater possibility of gene flow among closely related species. For example, gamete dispersal can move engineered genes from a target organism into a wild or domesticated relative more quickly and at a higher rate than might occur in hybridization via the movement of seeds among locations (O'Connor et al., 2015).

There are four broad types of movement that produce gene flow. First, individuals move via human-assisted dispersal. Human-assisted dispersal is well-recognized as a common avenue for the introduction of unwanted invasive species (Fonzi et al., 2015), but humans also move genotypes from one area to another. This can be accidental, as in the transport of marine organisms in ballast (Hershler et al., 2015) or purposeful, as in the enhancement of game or fishery populations (Anderson et al., 2014). Human-assisted movement can produce high or low rates of gene flow, depending upon the numbers of individuals transported. Second, individuals move in response to disruptive events. These can include evacuation in response to wildfires or other sources of rapid habitat destruction or fragmentation (Crosby et al., 2009; McElroy et al., 2011). Individuals in aquatic systems can also be transported among locations by flooding events such as flash flooding of streams or sheet flows across large areas (Apodaca et al., 2013). Gene

flow from disruptive events can occur at a high rate if the event does not also cause high mortality. Third, the life history of many species includes a significant probability of normal movement from one population to another without human assistance or a disruptive event (Graw et al., 2016). Rates of movement are highly variable, from cases in which it is rare for individuals to move to a different population to cases in which a significant fraction of the population disperses during every generation. Fourth, individuals can move in response to their perceptions of the quality of their current environment and that of nearby locations. For example, some animals will emigrate from a population in response to crowding, a shortage of breeding sites, or other indicators of habitat unsuitability (Clobert et al., 2004). If local habitats vary in quality, gene flow rates will be asymmetrical, with more animals leaving some populations than others and, conversely, some populations receiving more immigrants than others (Kawecki, 2004). A particularly important situation occurs when individuals emigrate from a population with a high density of individuals to a neighboring population with a low density, or into an area of suitable habitat that was previously not occupied by the species (Gauffre et al., 2014). For example, colonists may come from several different local populations and rapid recolonize an area in which a local population has been driven close to extinction (McCauley et al., 1995).

The spatial scale of movement is highly variable (Bohonak, 1999). Clearly, human-assisted dispersal can transport individuals for long distances and thereby link populations that might never exchange migrants via the typical movement patterns of individuals (Fonzi et al., 2015). Similarly, movements in response to disruptive events can also involve long distances. "Normal" movements have patterns and characteristic distances that are specific to individual species and their life histories (Ronce and Olivieri, 2004), and these can differ even among species occupying the same habitat (Nidiffer and Cortes-Ortiz 2015). At one end of the spectrum, there are species in which individuals move only very short distances in their lifetimes; when this is so, gene flow is restricted to low rates of exchange only among adjacent populations (Baer, 1998). At the opposite extreme, there are species in which individuals move considerable distances in a lifetime, which can create extensive dispersal to many other populations regardless of the distance separating them (Jue et al., 2015).

The implications of gene flow for gene drives

Regardless of the type or movement, the spatial scale, or the life stage in which it occurs, gene flow at a sufficient rate can cause populations to converge in gene frequencies (Slatkin, 1985). Of course, complete convergence will not occur because populations of limited size will experience random changes in gene frequencies that act counter to gene flow's otherwise homogenizing influence. It is important to note, too, that gene flow may cause maladapted genes to move between the subpopulations. If dispersal is a relatively weak force compared to selection, maladaptive genes will be removed by selection, similar to the removal of spontaneously occurring deleterious mutations that appear in the local gene pool. However, a distinctly different evolutionary outcome will occur if the rate of dispersal exceeds the strength of selection. Here, dispersal can cause a population decline because maladaptive genes are introduced into a subpopulation faster than they can be purged by selection (Bolnick and Nosil, 2007).

These concepts have ramifications for gene drives. As discussed above, gene drive mechanisms may be specifically designed to introduce maladaptive or even lethal genes into a target population, and the mechanism may itself override the effects of natural selection. Therefore, if a gene drive construct is introduced into one population, dispersal may facilitate its entry into another population. This spread may be beneficial if the intent is for the gene drive to affect multiple populations. However, if the intent of the gene drive is to affect a single target population, then gene flow may spread the gene drive to non-target populations, thereby creating unintended evolutionary and ecological consequences. If a gene drive construct reaches a non-target population, its fate will be governed in part by the fitness it imparts across genetic backgrounds and by its conversion rate. Conversely, if a gene drive is deployed for conservation goals, for example, to suppress the population of an invasive rodent on an island, the social system of the rodents may limit the ability of the introduced organisms carrying the gene drive to establish territories, obtain mates, and spread the desired gene through the population.

It is clear that knowing the amount and pattern of gene flow among populations will be crucial for predicting the spatial dynamics of a gene drive that is released in the environment (North et al., 2013). Some studies have begun to model more complex scenarios of population history (Deredec et al., 2008, 2011), but many features of gene drives can be modeled more explicitly. These include, but are not limited to: the effects of mixed mating systems (e.g., plants that self-fertilize and outcross at varying rates); the effects of spatial structure and gene flow; the potential for selection to act against migrants from another population if a drive is meant to spread spatially (Nosil et al., 2005); the evolution of resistance to the gene drive allele, which may lose effectiveness over time; the population dynamics of off-target effects (unintended editing of genes within the organism) that could lead to unexpected and undesired genetic changes; and the capacity for pathogens to overcome engineered resistance (as in the case of malarial resistance in mosquitoes). Additional modeling is necessary both for a more nuanced view of the capabilities and promise of gene drive, as well as for risk assessment (see Chapter 6 for further discussion).

Nonetheless, even the simplest models highlight important empirical shortcomings. For example, although empirical evidence indicates suggest that conversion rates for gene drives are high for specific wild-type alleles in the laboratory (Gantz and Bier, 2015; Gantz et al., 2015; Hammond et al., 2016), there are, as of yet, no estimates of gene drive conversion rates in larger and more genetically variable populations. There are additional challenges awaiting the study of the fitness benefits or costs because estimates based on assays of edited genes may not always reflect the benefits and costs created by the gene drive constructs, even in the laboratory. For example, Hammond et al. (2016) found that while heterozygotes for three genes edited to drive female fertility to zero in *Anopheles gambiae* showed no differences from the fertility of wild-type homozygotes, the heterozygotes for two of the edited genes formed by gene drive constructs had fertility rates so low that a gene drive construct using them would fail to increase in frequency. Heterozygotes for the third gene also had reduced fertility but Hammond et al. (2016) showed that the gene drive construct would still increase in frequency. It is difficult at present to model the spread of a gene drive without estimates of important model parameters, including fitness, conversion rate, population structure, gene flow, and ecological interactions among others. Empirical measurements of all of these important parameters are important prerequisites for the release of gene-drive modified organisms.

The potential for effects on non-target species: horizontal gene transfer

A related concern is that the release of gene-drive modified organisms may affect the evolution of species that are entirely distinct from the intended target species. Horizontal gene transfer (HGT; sometimes called lateral gene transfer) is similar to gene flow, but it refers to the movement of genes between populations of otherwise distinct species. There is increasing evidence that HGT has profoundly impacted the evolution of prokaryotes, because of multiple mechanisms that allow genes to be transferred between unrelated bacterial species (Koonin et al., 2001). This transfer facilitates introduction of novel DNA into the chromosomes of bacterial cells via infection of genetic elements (plasmids or phages) or simple uptake of DNA from the environment. In addition, HGT can allow genes to cross between biological domains (Bacteria, Archaea, Eukaryota), which constitute the highest taxonomic levels in biology and that are separated by billions of years of evolution (Hilario et al., 1993; Aravind et al., 1998; Klotz et al., 2003). This possibility is exemplified by *Agrobacterium tumefaciens* bacterial infection of plants that can permit genes to move from bacteria into the host plant genome (Krenek et al., 2015).

The existence of HGT creates the concern that gene drive mechanisms, or their individual component parts, may spread into non-target species. Although HGT may occur more slowly in an evolutionary sense than the production of genetic variation within a species, it has also been argued that HGT can exact more profound changes in natural populations, perhaps contributing to major evolutionary transitions (Gogarten and Townsend, 2005; Keeling and Palmer, 2008; Syvanen, 2012). There is also a growing appreciation that the likelihood of HGT events may vary among eukaryotic lineages, with the historical occurrence of these events perhaps being more common in plants than in other eukaryotes (Andersson, 2005). Moreover, separate but closely-related species of plants often hybridize (Rieseberg and Carney,

1998), suggesting that the possibility of the horizontal exchange of gene drives between species should need to be evaluated prior to environmental release.

Removal of Substantial Reduction of a Target Species

One possible goal of release a gene-drive modified organism is to cause the extinction of the target species or a drastic reduction in its abundance. Whether this outcome produces undesirable ecological consequences or not will depend upon factors that will vary from case to case.

The fundamental issue at the crux of ecological consequences of releasing a gene-drive modified organism is the fact that species do not exist in an ecological vacuum. Individual species are connected to other species in the community through direct trophic links (e.g., species A preys on species B) and through indirect trophic links (e.g., species C competes with species D for the same resource, or species E and F are both preyed on by species G). These links create dynamic feedbacks that affect the relative abundances of different species (Wootton, 1994). The feedback loops and their associated nonlinear dynamics can create a system of considerable complexity (Scheffer, 2009; Leroux and Loreau, 2010). This complexity makes accurate prediction difficult in the abstract because individual situations will vary; however, theory and empirical results offer insights about the issues that could come into play.

First, removing a species or substantially reducing its abundance can alter the community in which it is embedded. This is most obvious when a so-called keystone species[6] is removed. The most well-known examples are keystone predators, which are predators at the top of a food chain whose loss triggers a dramatic change in the abundance of species at all lower levels of the food chain (Paine, 1966; Estes et al., 2011).

Second, the impact of removing a species can depend on whether there are ecological equivalents in the community. A target species may be abundant because it out-competes its ecological equivalents and keeps their abundances low (Klatt et al., 2015). In such cases, removing the target species may produce a competitive release of the other species, and the increase in their abundance may compensate for the loss of the target species in terms of any wider effects on the community that might otherwise radiate through the food web.

Third, there is increasing evidence that communities have tipping points at which they change rapidly from one configuration to another (Scheffer, 2009; Travis et al., 2013). Tipping points and alternative stable states are characteristic of systems, including ecological communities that include non-linear dynamics and that contain multiple feedback loops. A system can move past a tipping point when the abundance of a critical species passes a threshold value; complete removal of a species is not necessary to send an ecosystem across a tipping point into a new mix of species and abundances (Bundy and Fanning, 2005). A critical feature of these alternative states is that, in some cases, it may be very difficult to push the system back to its previous configuration, even with active restoration efforts (Burkepile and Hay, 2008; Mumby and Steneck, 2008). To be sure, there have been successful restorations of ecosystems (Shapiro and Wright, 1984) but a successful reversal cannot be assumed possible and, even if probable, could require many years of sustained effort (Jyvasjarvi et al., 2013).

Whether the ecological consequences of species removal or reduction through releasing a gene-drive modified organism are considered "desirable" or "undesirable" will depend on the context. For example, in the most straightforward case, removing or reducing the abundance of a recent invasive species may facilitate the recovery of endangered populations and the restoration of much of a community that has been disrupted by the invader.

In another example, when suppressing a target species releases ecologically equivalent species that, in effect, replace the target species' role in the ecosystem, it is unlikely that there will be substantial additional effects that would be considered "undesired." However, it is possible that the release of ecological equivalents may vitiate the effect of suppressing a target species. This seems most likely when the target species is a vector for a pathogen that can also be transmitted by the ecologically equivalent competitors

[6]Any species whose effect on its ecosystem is disproportional to its relative abundance (Denno and Lewis, 2009).

that may be released (Rey and Lounibos, 2015). For example, several species of *Aedes* can transmit dengue and chikungunya, and suppressing the numerically dominant species may induce the release of the others (Alto et al., 2015).

Ecological principles suggest that the most likely scenario for creating an undesired ecological consequence via population suppression would be if a gene drive were to be deployed on a native keystone species (i.e., not a disruptive invasive species). At this time, few, if any, of the candidates for the deployment of gene drives represent known keystone species. Perhaps the most prominent candidates are mosquitos, the larvae of which are eaten by a variety of aquatic predators (Kumar and Hwang, 2006; Shaalan and Canyon, 2009) and the adults of which are considered by some to be a resource for bats (Salinas-Ramos et al., 2015). While there is evidence that some species of bats will alter habitat use to capitalize on swarms of adult mosquitoes (Gonsalves et al. 2013a), mosquitoes in general do not appear as an important component of bat diets except perhaps for very small bodied species (Jones et al., 2009; Gonsalves et al., 2013b).

While present discussions do not focus on native keystone species, future proposals may do so. There is also the possibility that a gene drive could have a non-target effect by moving into a species for which it was not intended via hybridization (Rieseberg and Ellstrand, 1993; Ellstrand, 2014; Kraus, 2015). In this light, it will be important to consider prospectively and carefully the likelihood of an undesired outcome. The biggest challenge is the rapidity with which gene drives can spread, because consequences could occur too quickly for any adaptive management scheme to halt them.

Many of these points were made in the Ecological Society of America's most recent report on genetically modified organisms in the environment (Snow et al., 2005). The conclusions and recommendations of that report can be applied to many of the ecological issues surrounding the release of gene-drive modified organisms, with the added emphasis on the with which a gene drive can spread and the possibility of rapid development harmful ecological consequences.

Evolutionary Considerations

Evolutionary biology suggests two additional considerations about assessing the potential ecological effects of gene drives, particularly when it is used to remove a a target species or reduce its abundance. The first is evolutionary history. Species interactions are often not merely ecological processes but evolutionary results (Kerr et al., 2015). This is most obviously true in pathogen-host systems (Duffy and Hall, 2008) and predator-prey systems (Brodie et al., 2002) in which the features of one population have been molded by its coevolution with a population of another species (Thompson, 2005). Disrupting a co-evolved system by removing one species can produce a dramatic effect in the other species. Whether this is considered undesirable depends, again, on context. In some cases, this is precisely the goal of deploying a gene drive construct: Suppressing a disease vector will have an adverse effect on the pathogen carried by that vector. On the other hand, if a predator has evolved specialized features that improve its ability to capture and consume an individual prey species, at a cost to its ability to consume other species, then removing the prey will have an adverse effect on the predator because it cannot readily switch its consumption to other species. While at present, there is no proposal for deploying a gene drive in such a context, it is possible that a gene drive could have a non-target effect of this type. This might be of particular concern in plant groups in which gene flow across species is possible and the effects of a non-target suppressor could translate into undesired effects on specialized insect pollinators and herbivores.

The second consideration is evolutionary future. Species that have been the targets of control mechanisms have often evolved some form of resistance that has allowed the recovery from the reductions in abundance produced by the initial application of those control mechanisms. The classic cases are antibiotic resistance (Perron et al., 2015), pesticides (Georghiou, 1990), herbicides (Busi et al., 2013), and viral control agents (Kerr et al., 2015). It is possible that resistance to a gene drive will arise. Resistance may evolve rapidly enough to impair the effectiveness of a gene drive for either population suppression or population modification, such as has been proposed for interfering with transmission of viral pathogens. Indeed, the lower the equilibrium population mean fitness becomes after the introduction of a gene drive,

the stronger the selection pressure will be on any beneficial resistance mutant that arises even though the rate of these mutation will be lower as well. For a gene drive, the resistance could be systemic (i.e., to Cas9) or could depend on the target gene (i.e., gRNAs). The evolution of resistance is not guaranteed because resistance might depend on specific characteristics of individual species such as the frequency of end-joining (NHEJ) DNA repair, or its timing, or the overall mutation rate, which can vary widely among species and even lineages within a species (Denver et al., 2012).

CONCLUSIONS

A wide variety of gene drives occur naturally in many types of organisms that cause genes or other genetic elements to spread throughout populations to varying degrees. To date, most gene drive research focuses on insects, although some research has also been conducted on yeast and mice. Preliminary evidence from research using mosquitoes, fruit flies, and yeast suggests that gene drives developed in the laboratory with CRISPR/Cas9 could spread a targeted gene through nearly 100% of a given population. Cell types and species are likely to differ in their capacity to carry a gene drive, and therefore the effects and efficacy of gene drives are expected to be largely species-dependent. Additional laboratory research is needed on CRISPR/Cas9 and other gene drive mechanisms, both to refine these approaches and to understand how they might work under different environmental conditions and in a diversity of organisms.

Research on the molecular biology of gene drives has outpaced research on population genetics and ecosystem dynamics, two fields of study that are essential to determining the efficacy of gene drives and their biological and ecological outcomes. There are considerable gaps in knowledge on regarding the implications of gene drives for an organism's fitness, gene flow in and among populations, and the dispersal of individuals, and how factors such as mating behavior, population sub-structure, and generation time might influence a gene drive's effectiveness. Addressing knowledge gaps about gene drives will require the convergence of multiple fields of study including molecular biology, genome editing, population genetics, evolutionary biology, and ecology.

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3

Case Studies to Examine Questions About Gene-Drive Modified Organisms

To examine the questions surrounding gene drive research, this report relies heavily on an extended, iterative exploration of a set of *plausible* case studies. The case studies are first described in a preliminary fashion in this chapter. Other chapters build on these case studies with deeper discussion of issues pertinent to value-based concerns, scientific techniques to mitigate harms, risk assessment, public engagement, and governance.

The case studies offer practical scenarios on which to base the report's analysis and recommendations and to provide a sound foundation for the further discussions that will necessarily follow this report as gene drive research advances. Given those two goals, the committee used the following three criteria to select case studies:

- Plausibility: Selection of organisms suitable for the development of a gene drive.
- Likelihood: Selection of areas for gene drive research or applications that are expected to be pursued in the near term.
- Diversity: Selections are intended to reflect a range of plausible target organisms, applications, mechanisms of action, and locations (in terms of where gene drive research is carried out and where organisms could potentially be released).

BASIC CRITERIA FOR THE DEVELOPMENT OF GENE-DRIVE MODIFIED ORGANISMS

It is particularly important to understand what is meant by *plausibility*. Many organisms and traits are not suitable for gene drive research. The two most basic requirements for a target organism of gene-drive work are that it reproduces sexually and that it reproduces rapidly (see Box 3-1). For this reason, many insects and rodents are good candidates for gene drive research. Organisms such as viruses, many plants, and most bacteria, which use other means to reproduce, are not good targets for gene drive research (see Box 3-2 for additional considerations for plants). Humans, elephants, and trees are also not good targets for gene drive research because they have long generation times; any modification introduced into such a population could require decades or centuries to become established. However, a gene drive could work in an organism that has alternating sexual and asexual phases of reproduction, as in *Plasmodium falciparum*, the parasite that causes malaria (de Koning-Ward et al., 2015), even though its population structure may render spread of the gene drive difficult.

BOX 3-1 Basic Criteria for the Development of Gene-Drive Modified Organisms

1. Sexual reproduction
2. Relatively short generation time
3. Stability of the driving genetic elements
4. Population structure appropriate to the desired outcome.

BOX 3-2 Additional Considerations for Gene-Drive Modified Plants

Plants vary considerably with regard to the four primary criteria for the creation of gene drives described in Box 3-1. For example, some plants commonly reproduce sexually; however, the model species rice and *Arabidopsis thaliana* rarely outcross and are therefore unlikely to be reasonable choices for gene-drive approaches. Plants also possess different generation times; gene drives will proliferate more rapidly in annual or biennial plants compared to long-lived species. The role of population structure, which could limit the spread of the gene drive if individuals are not available geographically to reproduce, is also important to consider. Population structure could be detrimental if the goal is to propagate a gene drive throughout an entire species (although this could be overcome by multiple releases), but it may be useful if the intent is to constrain a gene drive to a particular locality. Finally, plants have the potential for a particular form of genetic structure called soil seed banks, which contain seeds waiting for the right environmental signal(s) for germination. Many plants have seeds that remain dormant in the soil for tens to hundreds of years, providing a genetic repository that cannot be reached by a gene drive until the seeds finally germinate and reproduce.

In addition, some traits may simply be too complex to alter because they are governed by many genes, their expression is shaped by the external environment, or they are modified by internal or development cues (e.g., epigenetics) that are not yet fully elucidated. For example, flowering time in maize is determined by the cumulative effects of many genes (Buckler et al., 2009).

In some case, many applications of gene drive research may not be necessary, because efficient non-gene drive approaches are able to generate the desired outcome.

Given these and other technical and regulatory challenges (discussed in detail in the other chapters), predictions about how gene drives might be used need to be treated critically. The committee developed case studies to illustrate the issues highlighted in Table 3-3.

CASE STUDY 1: USING *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* MOSQUITOES TO MANAGE DENGUE

Objective

Establish gene drives in *Aedes aegypti* and *Aedes albopictus* mosquitos to control the spread of dengue throughout the world.

Rationale

Dengue, a debilitating viral infection, is one of the leading causes of sickness and death in subtropical and tropical countries around the world. Adults and children who contract dengue often experience a flu-like illness. Severe dengue, also called Dengue Hemorrhagic Fever, causes bleeding, persistent vomiting, breathing difficulties, and other complications that may lead to death. Severe dengue disproportionately affects children.

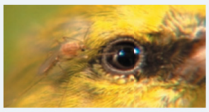
Dengue is caused by infection with any of five serotypes of dengue virus (which is a flavivirus). The virus is transmitted to humans via the bite of female[1] *Aedes aegypti*, the primary vector (carrier) in urban areas, or *Aedes albopictus*, the primary vector in rural areas. In April 2016, the World Health Organization endorsed the use of the first-ever dengue vaccine, Dengvaxia (CYD-TDF) by Sanofi Pasteur, in countries where dengue is endemic.[2] Research is ongoing for other vaccine candidates. Patient recovery for those who are unvaccinated depends heavily on an early diagnosis and careful management of fever symptoms.

[1]Only female mosquitoes bite and drink blood. Female mosquitoes need the protein in blood to make their eggs.

[2]http://www.who.int/immunization/research/development/dengue_vaccines/en [Access May 2, 2016].

Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values

TABLE 3-3 Selected Case Studies on Gene Drive Research and Related Applications

Public Health		
1	Use the mosquitoes *Aedes aegypti* and *Aedes albopictus* to manage dengue	 Aedes aegypti Credit: James Gathany
2	Using *Anopheles gambiae* mosquitoes to combat malaria	 *Anopheles gambiae* Credit: James Gathany
Conservation		
3	Using the *Culex quinquefasciatus* mosquitoes to combat avian malaria	 Mosquito biting a honeycreeper Credit: Susanne Bard
4	Controlling populations of non-indigenous *Mus musculus* mice to protect native biodiversity on islands throughout the world	 *Mus musculus*
5	Controlling non-indigenous *Centaurea maculosa* knapweeds to protect biodiversity in rangelands and forests in the United States	 *Centaurea maculosa* Credit: Matt Levin
Agriculture		
6	Controlling *Amaranthus palmeri* (Palmer amaranth, also known as pigweed) to increase agriculture productivity in the Southern United States	 *Amaranthus palmeri* Credit:Michael J. Plagens
Basic Research		
7	Developing a vertebrate model for gene drive research using *Danio rerio zebrafish*	 *Danio rerio* Credit: Monte Westerfield

Current Mitigation Efforts

Prevention of dengue relies entirely on vector control, mostly through ultra-low volume spraying of insecticides. Insecticide resistance is challenging the efficacy of such dengue vector control methods using currently available chemicals. Another vector control intervention is the management of mosquito vector breeding sites, which are typically man-made containers. However, because dengue disease exhibits spatiotemporal heterogeneity epidemic activity (alternating as high and low incidences between years and seasons), and because of the potential serotype interaction and co-circulations, predicting possible epidemics is extremely complex as is effective prevention. These strategies are laborious and typically reactive rather than proactive (Achee et al., 2015). Additional control strategies are listed in Appendix C.

Biological controls also exist, such as the use of cyclopoid copepods (Marten et al., 1994), population reduction via community participation (Scholte et al., 2006; Majambere et al., 2007) and the use of larvivorous fish, but the maintenance of the distributed containers is a limiting factor to effective control. Another type of biological control is through the release of *Wolbachia*-infected mosquitoes. The bacterial symbionts in the genus *Wolbachia* are widely distributed in insects (Werren, et al., 1995, Werren and O'Neil, 1997; Bourtzis and Braig, 1999;Stouthamer et al., 1999). *Wolbachia* infection reduces the lifespan of the insect hosts (Sinkins et al., 1997; Dobson et al., 2002; Ahantarig et al., 2011; Bull and Turelli, 2013). In addition, *Wolbachia* infection of *Aedes aegypti* confers resistance to infection with dengue and chikungunya viruses (Bian et al., 2010; McMeniman et al., 2009; Moreira et al., 2009). In light of these results, small-scale trials to reduce dengue transmission using *Wolbachia* started in 2011 in Australia and further expanded to Vietnam, Indonesia, and Brazil.[3] Although on-going large field trials suggest a reduction of dengue incidence, there remain important considerations concerning the unanticipated evolution of the dengue virus (or other viruses infecting the same mosquito vector) that need to be addressed.

In summary, despite many available methods of mosquito control, existing methods are not yet fully effective at reducing dengue transmission.

Plausibility of a Gene Drive Solution

It may be possible to create two types of gene drives in *Aedes* species: one that prevents the transmission of the dengue virus and another that causes sterility. Research with *Wolbachia* demonstrates, in principle, the potential for those two approaches. In 2010, researchers showed that *Wolbachia* can be used to induce resistance in *Aedes aegypti* to the dengue virus. *Wolbachia* also can be used to shorten the lifespan of *Aedes aegypti* (McMeniman et al., 2009). Similarly, the U.K.-based company Oxitec has developed a technology to suppress *Aedes aegypti* populations in which male *Aedes aegypti* mosquitoes are genetically engineered to be sterile.[4] The first proofs-of-concept experiments demonstrating the creation of a gene drive in the fruit fly, a model organism for invertebrate research, and in other mosquito species (discussed below) also provide evidence that a gene drive could be developed in *Aedes aegypti* (Gantz and Bier, 2015; Gantz et al., 2015; Hammond et al., 2016). These applications would require initial release of a number of the gene-drive modified mosquitoes within an urban setting where dengue is endemic or where dengue outbreaks are known.

CASE STUDY 2: USING *ANOPHELES GAMBIAE* MOSQUITOES TO COMBAT HUMAN MALARIA

Objective

Create gene drives in *Anopheles gambiae* mosquitoes to reduce the spread of human malaria in Sub-Saharan Africa.

[3]For details, see http://www.eliminatedengue.com/progress.

[4]http://www.oxitec.com/health/our-solution/ [Accessed February 16, 2016].

Rationale

Malaria is a serious and sometimes fatal parasitic infection that occurs in nearly 100 countries worldwide. Adults and children who contract malaria often experience high fever and anemia. If the infection is severe, coma and death can occur. Malaria disproportionately affects people, particularly children, in low and middle income countries in sub-Saharan Africa, South Asia, and South America.

Human malaria is caused by any of the five protozoan parasites of the *Plasmodium* genus. The mosquito *Anopheles gambiae* is the primary vector (carrier) of *Plasmodium* in Sub-Saharan Africa.

Current Mitigation Efforts

Current methods for malaria control focus on two themes, drug therapy and vector control. The ability to treat infection requires detection of the parasite and access of infected persons to healthcare, which can be extremely challenging in many, if not most, malaria-endemic settings. Malaria vaccines are under development and have shown promise, but will take many more years before they can be fully recommended for wide application. Prevention of transmission targeting the Anopheline mosquito vector is based on interventions recommended by the World Health Organization. These include measures to eliminate breeding sites, spraying insecticides with residual properties onto the walls of houses, and using insecticide-treated bed nets in areas where malaria is endemic. Additional control strategies are listed in Appendix C. However, all of these measures require organized campaigns and sustained resource availability. In addition, efforts to control malaria are in jeopardy due to the spread of insecticide resistance in *Anopheles gambiae* populations (Edi et al., 2012; Namountougou et al., 2012; Cisse et al., 2015).

Plausibility of a Gene Drive Solution

A gene drive that alters the female mosquito's ability to become infected with the malaria parasite, or one that prevents parasite development within the mosquito, could block malarial transmission without affecting mosquito populations. In November 2015, researchers demonstrated that CRISPR/Cas9 can be used to create a gene drive that could spread anti-*Plasmodium* genes in populations of a malaria-carrying *Anopheline* mosquito, *Anopheles stephensi* (Gantz et al., 2015). However, the system transmits the drive construct at Mendelian frequencies in some crosses, suggesting that this valuable proof-of-principle needs further modification and research before field release (Gantz et al., 2015). Alternatively, a gene drive that alters the fitness of the female mosquito could result in reducing vector populations over time. In December 2015, researchers demonstrated that CRISPR/Cas9 can be used to create a gene drive that causes sterility in female *Anopheles gambiae* mosquitoes (Hammond et al., 2016). Although one of the research team's constructs is predicted to spread through a population, it has not yet been shown to spread to high frequency in a population containing heterogeneous genetic backgrounds. Nonetheless, the anti-*Plasmodium* and the female sterility gene-drive approaches theoretically have the potential to eliminate malaria in Sub-Saharan African villages where malaria is endemic.

CASE STUDY 3: USING *CULEX QUINQUEFASCIATUS* MOSQUITOES TO COMBAT AVIAN MALARIA IN HAWAII

Objective

Create gene drives in southern house mosquitoes, *Culex quinquefasciatus*, to reduce the spread of avian malaria to threatened and endangered honeycreeper birds in the Hawaiian Islands.

Rationale

Avian malaria is a disease caused by protozoan parasites that infect birds. Birds become infected when they are "bitten" by female mosquitoes carrying the parasite. Birds without immune resistance to the parasite become anemic, grow progressively weaker, and ultimately die. Avian malaria is common in most continents, but absent from many isolated islands where mosquitoes (and hence *Plasmodium*) do not naturally occur (Atkinskon, 2005).[5] Thus, native birds in Hawaii, the Galapagos, and other archipelagoes, which evolved without natural exposure to *Plasmodium* parasites, are highly susceptible to avian malaria. The southern house mosquito, *Culex quinquefasciatus***,** is the primary mosquito vector of *Plasmodium relictum* in Hawaii.The displacement and extinction of native birds has greatly impacted ecological systems and biodiversity in Hawaii, and climate change threatens to expand mosquito ranges into higher elevations, thereby presenting greater harm to bird populations at these elevations.

Current Mitigation Efforts

Prevention of avian malaria transmission has historically been through interventions that target mosquito vector populations using insecticide spraying and larval source management. Similar to resistance of parasites to drugs, many mosquito species are resistant to currently available chemicals, making control difficult. In Hawaii, attempts to control the mosquitoes through such methods have not eliminated the threat. See Appendix C for a comprehensive list of mosquito control strategies.

Plausibility of a Gene Drive Solution

The use of gene drives could be used as a new strategy to target the mosquito vector to control avian malaria. As described in the first two case studies, there is strong potential to develop gene drives that alter the female mosquito's ability to become infected with the malaria parasite, or that prevent mosquitoes from reproducing. The first proofs-of-concepts in which gene drives were created in the fruit fly and in other mosquito species provide evidence that a gene drive could also be developed in *Culex quinquefasciatus* (Gantz and Bier, 2015; Gantz et al., 2015; Hammond et al., 2016).

CASE STUDY 4: CONTROLLING POPULATIONS OF NON-INDIGENOUS *MUS MUSCULUS* MICE TO PROTECT BIODIVERSITY ON ISLANDS

Objective

Reduce or eliminate populations of the non-indigenous mouse, *Mus musculus*, to protect native biodiversity on islands around the world.

Rationale

Invasive species are a leading cause of extinction of native wildlife and plants on islands. Nearly half of all species included on the International Union for the Conservation of Nature's list of species that are threatened with extinction live on islands. In addition, roughly 70%, 90%, and 95% of all extinctions of mammals, reptiles, and birds occur on islands, respectively (Campbell et al., 2015; Godwin, 2015). The activities of the house mouse, *Mus musculus*, and other introduced rodents reduce the ability of native species to reproduce, alter or destroy habitats so that they no longer support the needs of native species, and in other ways negatively affect island ecosystem dynamics. Approximately 80% of the world's islands now have invasive rodents (Campbell et al., 2015; Godwin, 2015).

[5]https://pubs.usgs.gov/fs/2005/3151/report.pdf.

Current Mitigation Efforts

Efforts to eradicate rodents from islands include the use of traps, poisons, and biological controls, such as the introduction of predators or diseases. Application of rodenticides can be cost-prohibitive due to expenses associated with regulation compliance, dispersal method, size of the treated area, and cost of the toxicant itself (Meerburg et al., 2008; Williams, 2013). Mechanical traps are often considered more humane than rodenticides because they do not involve the use of chemicals that could adversely affect human, animal, and overall ecosystem health (Lorvelec and Pascal, 2005; Witmer et al., 2011). However, placing traps and collecting the caught animals is labor intensive, traps do not discriminate between target and non-target organisms (Lorvelec and Pascal, 2005), and traps are insufficient to fully eradicate a rodent population without the use of other methods. Other research aims to use genetic engineering approaches to control rodent populations including RNA interference and developing transgenes that cause female progeny to develop as males or prevent all progeny from developing (Gemmell et al., 2013; He et al., 2015). It remains to be seen if such genetic engineering approaches will be effective, scalable and affordable (Campbell et al., 2015; Jacob et al., 2008). Additional discussion of these methodologies and a more comprehensive list of other approaches used to control rodent populations are presented in Appendix D.

Plausibility of a Gene Drive Solution

Scientists are studying a sex-determining gene drive that causes house mice to produce more male offspring than females (Cocquet et al., 2012). If this occurs over multiple generations, it should lead to a reduction in population size over time. The molecular mechanism takes advantage of an endogenous region of high meiotic drive (meaning it is more likely to be inherited) in the mouse genome found on chromosome 17 (an autosome) called the t-complex. In this scenario, male mice are genetically engineered to possess the *Sry* gene, which promotes male characteristics (Goodfellow and Lovell-Badge, 1993), on chromosome 17 instead of its usual location on the Y chromosome. An XY Sry male is fertile, and upon mating to a wild-type XX female, both the XY and XX offspring (both male and females) possess Sry and physically develop into male mice, with XX male mice being sterile and the XY mice still able to reproduce and transmit Sry. Over time, the population of mice would tend to become all male, leading to a decrease in reproduction and eventual population decline and suppression due to the loss of female mice (Campbell et al., 2015). Male mice are promiscuous, and so have nearly an unlimited amount of reproductive potential, as long as fertile female mice are present. Female mice must go through a gestation period after mating, limiting their ability to contribute their genetic information to future generations. Hence, female mice are the limiting factor in the change of population densities over time. A description of the technique, and elements that helped in the development of a case study in this report can be found on a website dedicated to island conservation created by students from North Carolina State University.[6]

Other potential gene-drive mechanisms based upon Medea or underdominance strategies could also be used to achieve the same purpose and would involve inducing targeted translocations into the mouse genome.

CASE STUDY 5: CONTROLLING NON-INDIGENOUS *CENTAUREA MACULOSA* KNAPWEEDS TO PROTECT BIODIVERSITY IN RANGELANDS AND FORESTS

Objective

Create gene drives in the non-indigenous knapweed species, *Centaurea maculosa*, to protect biodiversity of native plant species in rangelands and forests in the United States.

[6]https://research.ncsu.edu/islandmice/what-can-genetic-engineering-offer/how-is-this-strain-created/.

Rationale

The spotted knapweed (*Centaurea maculosa*) is native to Eastern Europe but was introduced to the United States in the late 1800s. By the year 2000, spotted knapweed could be found in 45 of the 50 states and covered nearly 7 million acres of rangeland and pine forest (Zouhar, 2001). Spotted knapweed first invades disturbed habitats; once established, it spreads to native ecosystems, causing soil erosion in the process.

Current Mitigation Efforts

Several attempts have been made to slow the spread of spotted knapweed by using biological controls; these reduce seed production but have not had large effects on the density of *Centaurea maculosa* plants (Sheley et al., 1998). In addition to biological controls, management of knapweed populations has focused on physical removal, fire, and chemical treatment for infestations (Sheley et al., 1998; Zouhar, 2001).

Plausibility of a Gene Drive Solution

Spotted knapweed is obligately outcrossing (Harrod and Taylor, 1995), meaning that there is little or no self-fertilization and that gene drives would be able to spread throughout knapweed populations. Another factor that makes it potentially suitable for a gene drive is that the basis for its ability to outcompete native plants is thought to come from the production of a compound called catechin (Thelen et al., 2005), which it exudes from its the roots. Catechin inhibits the germination and growth of native plant species, thereby conferring a competitive advantage to spotted knapweed (Bais et al., 2003).

There are two possible gene-drive approaches to help limit the spread of spotted knapweed, which could potentially be employed together. The first option is to engineer a suppression gene drive by targeting sex-specific genes, thereby biasing gender ratios and facilitating a population crash. The second is to modify the population by targeting the catechin biosynthetic pathway, which in theory would negatively affect the knapweed's ability to compete against endemic plants, although this effect is still debated (Perry et al., 2005). In either case, the rate of spread of either of these gene drives is expected to be slow, because spotted knapweed is a perennial plant that lives for approximately nine years (Zouhar, 2001). In addition, the success of a suppression drive is likely to depend critically on the fertility advantages of sex-modified plants compared to hermaphrodites and also on features such as pollen availability and spatial structure (Hodgins et al., 2008).

CASE STUDY 6: CONTROLLING PALMER AMARANTH TO INCREASE AGRICULTURE PRODUCTIVITY

Objective

Create gene drives in Palmer amaranth (*Amaranthus palmeri* also called pigweed), to reduce or eliminate the weed on agricultural fields in the Southern United States.

Rationale

Palmer amaranth infests agricultural fields throughout the American South. It has evolved resistance to the herbicide glyphosate, the world's most-used herbicide (Powles, 2008), and this resistance has become geographically widespread.

Current Mitigation Efforts

Whether a plant is considered a weed is context-dependent. In one region, a plant is desirable, whereas in another place, the same plant may be a weed. A plant is typically viewed as a "weed" when it has little recognized value in the locale where it is growing and when it grows rapidly and competes with a crop or pastureland for space, light, water, and nutrients. Weed management is a continual and major challenge. In addition to competition for resources and interfering with the management of desirable plants, poisonous weeds can negatively impact human health, crops and livestock (Bridges et al., 1994). Management strategies fall into four major categories: physical and mechanical methods, cultural methods, chemical methods, and biological methods. Examples of mechanical practices include manual removal of weeds, which is labor intensive, or tilling, which can increase soil erosion. Examples of cultural practices include crop rotations using plants that choke out weeds (often there are limited choices available) and using drip irrigation to limit water to planting rows, which only works well in dry regions that extensively irrigate. Examples of biological methods include animal grazing and the use of natural enemies (microbes, insects, and other animals such as nematodes, fish, and birds); these strategies are primarily used in low-intensity management of rangelands, forests, preserved natural areas, and waterways.

In much of production agriculture, the primary approach to control weeds is to use herbicides. Glyphosate, the most commonly used herbicide, is a systemic herbicide that, when applied, moves throughout the plant thus destroying more tissues as compared to contact herbicides. The generation of herbicide-resistant crops has revolutionized weed control. Glyphosate-resistant crops have been rapidly adopted in multiple crops because of economic advantages, strong weed control, and the observation that the glyphosate-resistant crop system confers a lower environmental impact than the approaches it replaced (Duke and Powles, 2009). Unfortunately, after decades of glycophosate use weeds are now adapting, and herbicide resistance is increasing among weed population, reducing the efficacy of glyphosate for weed control (Powles and Yu, 2010). The current strategy to deal with herbicide-resistant weeds is to adopt diverse tactics, combining multiple weed control approaches (Duke and Powles, 2009; Norsworthy et al., 2012). The particular combinations of strategies chosen depend on the crop, the region, and the major weeds impacting the particular agricultural system. Details on specific practices can be found on agricultural extension websites at land grant institutions throughout the United States and at equivalent international institutions' websites.

Plausibility of a Gene Drive Solution

Palmer amaranth is a likely candidate for gene-drive technology, for five reasons. First, it is an annual plant, so it has yearly sexual reproduction and a rapid generation time. Second, Palmer amaranth and some other members of the genus are dioecious (male and female flowers occur on separate plants) (Steckel, 2007), which ensures the outcrossing necessary to spread gene drives. Third, it does not have an extensive seed bank; studies suggest that most seeds do not persist in the soil, so that there is unlikely to be a seed repository that is immune to the gene drive. Fourth, an *Amaranthus* species has been transformed genetically (Pal et al., 2013), suggesting that it will be technologically feasible to insert gene drives into Palmer amaranth. Finally, Palmer amaranth is wind-pollinated, implying that the eradication of species will, at the very least, not harm insect pollinators.

In theory, Palmer amaranth could be subjected to two types of gene drive. In the first, a modification drive would target the genes that confer resistance to glyphosate and reestablish the population's susceptibility to glycophosate herbicides. The potential targets of this gene drive are known, because the glyphosate herbicide acts by interrupting the function of 5-enolpyruvylshikimate-3-phosphate synthase. In Palmer amaranth, this synthase gene has been duplicated extensively, leading to enzyme overexpression and glyphosate resistance (Gaines et al., 2010). Thus, a candidate gene drive would need to target multiple 5-enolpyruvylshikimate-3-phosphate synthase copies that are scattered throughout the genome. If the gene drive succeeded and susceptibility became fixed, glyphosate could then be used again as a tool to limit Palmer amaranth populations.

A second approach would be to build a suppression drive. Although the target and content of such a drive is not yet clear, the fact that there are separate male and female plants implies that there are sex-specific genes that are suitable targets for biasing the sex ratio. Under this approach, the goal would be skew sex ratios until the entire population (or species) collapses.

CASE STUDY 7: DEVELOPING A VERTEBRATE MODEL FOR GENE DRIVE RESEARCH USING ZEBRAFISH[7]

Objective

Create gene drives in the zebrafish, *Danio rerio*, to study gene-drive mechanisms in a vertebrate animal.

Rationale

As of April 2016, researchers have not developed a gene-drive modified vertebrate for use in fundamental research in the laboratory but proofs-of-concept for gene drives have been demonstrated in yeast, the fruit fly, and mosquitoes, with the expectation that this technique will be translated to a vertebrate animal at a future date (Gantz and Bier, 2015; DiCarlo et al., 2015; Gantz et al., 2015; Hammond et al., 2016). These current animal models, and the behavior of gene drives in them, will not necessarily recapitulate the behavior of gene drives in vertebrate species. Given the fundamental differences between vertebrates and invertebrates, a vertebrate species for gene drive research will be needed to address a variety of fundamental research topics before using gene drives in other vertebrate animals, particularly those intended for release into the environment; and also potentially to make comparisons with gene drive mechanisms in invertebrates.

Current Mitigation Efforts

Containment of zebrafish is straightforward due to the requirement for appropriate aquatic facilities, while other potential vertebrate models for gene drives, such as the mouse, could more easily escape from, and survive outside, the laboratory. In addition, it may be possible to develop a self-limiting gene drive in zebrafish by making the drive active only in the presence of tetracycline, which could be required to activate the promoter needed to express the gene drive construct (Hammond et al., 2016).

Plausibility of a Gene Drive Solution

A gene-drive modified zebrafish could be developed specifically for laboratory studies with no intention for environmental release. The zebrafish provides an outstanding model to address basic research questions about gene drives in a vertebrate species for many reasons (Shah and Moens, 2016). The zebrafish genome has been fully sequenced, and zebrafish have well-characterized traits associated with reproduction and other behaviors (Howe et al., 2013). Zebrafish are also low cost and easy to maintain, have a short generation time, and produce large numbers of offspring (Lawrence et al., 2012; Harris et.al,

[7]A mouse could also potentially be a candidate vertebrate model for gene drive research. Research on the naturally occurring t-complex in mice offers insight into how regions of high meiotic drive function and affect characteristics associated with vertebrate development and behavior (see Case Study 4). However, these studies may not be broadly applicable to other vertebrates. Also, the gestation period, and thus the generation time, is longer in mice than in zebrafish, which could make it more difficult for research to keep pace with rapid advances in invertebrates. However, existing approaches for gene editing through transient introduction of CRISPR/Cas9 (or other mechanisms) have been successful; thus, the committee considers development of a gene-drive modified mouse for laboratory research plausible, a close second to the case study on zebrafish presented in this report.

2014). They are also preferred from a regulatory standpoint (e.g., from the standpoint of Institutional Animal Care and Use Committee) with regards to using animal models for research. Moreover, gene editing has already been used successfully in this organism (Ma and Liu, 2015; D'Agostino et al., 2016; Lin et al., 2016; Prykhozhij et al., 2016).

A gene-drive modified zebrafish could be created by inserting a gene drive construct into the fish consisting of Cas9, a gRNA targeting a non-essential locus (e.g., a gene expressed in the eye) and a green fluorescent protein marker to identify the gene-drive modified organism. The latter characteristic would give rise to a visible phenotype upon insertion of the donor template on the construct.

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4

Charting Human Values

Why should we consider developing gene-drive modified organisms and releasing them into the environment? How should we select sites where such organisms could be released? How should we assess the outcomes? Do we need additional oversight mechanisms to govern gene drive research and development? These and many other questions underlie discussions within the scientific community and broader society about gene drives. Because gene drives are designed to alter the environments we share, in ways that might turn out to be very hard to anticipate and impossible to reverse completely, these questions are very complex and require careful exploration. The answers depend on *values*—deeply held, complicated, sometimes evolving beliefs about what kinds of things, in human lives and the world at large, should be fostered, protected, or avoided, and therefore about what people should and should not do (Elliott, 1992; Macrina, 2014). Values are critical components of human identity and society. They permeate our perceptions, understanding, hopes, fears, decisions, and actions. They are reflected in our views about what morality requires of us and in our views about what is in our interests, both individually and as a society. Values sometimes find expression in the sets of ethical principles formulated to guide science and medicine (Elliott, 1992; Macrina, 2014), such as the requirement that medical research on human subjects provide a positive balance of benefits over harms, the harms of participation are not borne disproportionately by disadvantaged or vulnerable people while the benefits go to those in positions of power and privilege, and that research no be conducted without the voluntary, informed agreement of the subjects (National Commission, 1978; WMA, 2013). Such values are understood to be important enough that they need to be treated not just as conventions but as obligations that can be enforced through a system of governance. Values are also the starting point of any attempt to decide what to do with emerging technologies. The committees and commissions charged with those decisions, identifying principles and making recommendations where possible, are engaged in the task of trying to articulate and sort through the implications of values (President's Commission, 1982; Presidential Commission 2010).

This chapter focuses on the values involved in gene drive research. The chapter begins with a brief overview of the scholarly debate that has unfolded over the last few decades about genetic engineering. Using the case studies presented in chapter three, the committee explored in depth three broad categories of concern:

- The potential benefits and harms of gene drive research for people,
- The potential impact of gene-drive modified organisms on the environment (understood both in terms of outcomes for people and, for some individuals and cultures, as a concern about the environment in its own right), and
- Who will be affected by gene drives and make decisions about them.

The exploration of these questions provides a conceptual framework for decisions about whether and how to move forward with the science and what kinds of constraints are appropriate in making decisions about field release. The chapter thus provides a conceptual underpinning for the specific recommendations found in later chapters.

CENTRAL VALUE CONSIDERATIONS IN DEBATES ABOUT GENETIC ENGINEERING

Genetic engineering sparked ethical debate as soon as it was imagined. Initially, in the 1960s, public debate focused on the prospect of using genetic engineering on humans; the possibility that genetic engineering might be a new and acceptable way of producing better human beings was exciting to some people and raised questions about eugenics for others. In the early 1970s, as scientists developed the ability to produce recombinant DNA, some of the researchers at the forefront of the work began to ask questions about the safety and environmental impact of the new molecules. At that time the questions focused chiefly on toxicity (Macrina, 2014). But as scientists learned how to produce a variety of genetically engineered organisms—primarily agricultural plants and animals at first, and later with the emergence of "synthetic biology," microbes that could be used in industry—critics raised additional questions about environmental, public health, and social effects (Presidential Commission, 2010). Just as gene-drive technology builds on earlier kinds of genetic engineering, ethical debates about gene drives are likely to build on these earlier considerations.

The most prominent moral questions about genetic engineering have always been about its prospective benefits and harms to human beings. The guidelines developed at the 1975 Asilomar Conference on Recombinant DNA focused on ensuring safety in the handling of potential biohazards (Berg et al., 1975). The seminal report *Splicing Life: The Social and Ethical Issues of Genetic Engineering with Human Beings*, issued in 1982 by the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, identified "balancing present and future benefits and risks" as the overarching ethical and social question that would have to be answered to decide whether and how to use genetic engineering technology (President's Commission, 1982). In a 2010 report on the ethical issues of synthetic biology, the Presidential Commission for the Study of Bioethical Issues identified "public beneficence" as the first of five "ethical principles" that should be used to assess synthetic biology and other emerging technologies (Presidential Commission, 2010). For decades, U.S. regulation of crops produced using genetic technologies has focused on questions of safety to consumers (under regulations enforced by the U.S. Food and Drug Administration), possible harms to other crops or plants in the environment (regulated by the U.S. Department of Agriculture) and the safety for humans and the environment of any pesticides that the plant may be engineered to produce (under regulations enforced by the U.S. Environmental Protection Agency).

A second set of questions turns attention away from defining the potential human benefits and harms to discussions about *who* will benefit or be harmed and *who* will make decisions about genetic engineering. In its 1982 discussion of human genetic engineering, for example, the President's Commission addressed parents' rights and responsibilities to make decisions about how genetic engineering might be used on their children, a general societal commitment to equality of opportunity, and to "a more basic question about the distribution of power: Who should decide which lines of genetic engineering research ought to be pursued and which applications of the technology ought to be promoted?" (President's Commission, 1982). The Commission argued that, in most cases, the public could rely on "the judgment of experts in the field" (President's Commission, 1982). However, in the Presidential Commission's 2010 report on synthetic biology, the thinking had changed: The Presidential Commission argued for the "intellectual freedom and responsibility" of experts in the field, but also insisted on "justice and fairness" in "the distribution of benefits and burdens across society," and it called for a principle of "democratic deliberation." The 2010 report argued that because biotechnology would affect the public, the public should participate "both in the development and implementation of specific policies as well as in a broader, ongoing national conversation about science, technology, society, and values" (Presidential Commission, 2010).

Third, and finally, the arc from the President's Commission in 1982 to the Presidential Commission in 2010 reveals a set of questions that are less easily articulated but are sometimes very deeply felt and have often been important in the public's reception of genetic technologies. The central theme in these questions is the possibility that some ways of using genetic technologies conflict with underlying moral norms that are implicit in how human beings understand the world, including their own nature and relationship to the rest of the world. In 1982 the President's Commission considered, and dismissed, a variety of objections to the

very idea of "splicing life," such as that it would usurp powers properly left to God (p. 53) or would constitute an "arrogant interference with nature" (p. 55). In 2010, the Presidential Commission agreed that engineering a genome is not intrinsically wrong: "After careful deliberation, the Commission was not persuaded by concerns that synthetic biology fails to respect the proper relationship between humans and nature" (p. 139). It allowed, however, that the use of that power should adhere to a principle of "responsible stewardship," and it elaborated this principle as a responsibility to be good "stewards of nature, the earth's bounty, human health and well-being, and the world's safety" (p. 123). This way of talking about stewardship leaves some room for asking questions about the human relationship to nature: Although genetic engineering can be consistent with social standards for the human relationship to nature, using it to destroy significant natural phenomena might not be. Moreover, it might not be responsible even if the destruction of those natural phenomena were consistent with human health and well-being.

All three of these broad kinds of value considerations are raised by research into gene drives. There are significant potential benefits and harms for humans. There are also questions about who would benefit, who would be harmed, and who would be empowered to make decisions about gene-drive technologies. Additionally there are significant potential environmental benefits and harms, and how to understand the values relevant to the potential environmental outcomes can be challenging. Although other genetic technologies have raised questions about environmental outcomes, the power of a gene drive to alter an entire population or species, perhaps even to bring about the local or global eradication of a species, is a meaningful expansion of the human capacity to alter the shared environment (Esvelt et al., 2014; Oye et al., 2014; Caplan et al., 2015; Webber et al., 2015). It raises questions about both public health and about the human relationship to nature.

POTENTIAL HUMAN BENEFITS OF GENE DRIVES

The primary rationale for pursuing research on gene drives is the hope that it might produce human benefits. The potential human benefits envisioned in the case studies presented in Chapter 3will be significant to many people. The potential public health benefits are particularly promising, but agricultural benefits may also be possible. Given the early stage of the research, as-yet-unrealized benefits may become evident as the science develops. For many researchers, the possibility of uncovering new kinds of benefits and of gaining new scientific insight itself can be important motivating factors.

Potential Public Health Benefits

Creating gene drives in mosquitoes to combat infectious diseases like dengue and malaria (Case Studies 1 and 2) holds potential public health benefits, particularly the control of arthropod vectors, such as insects and ticks. Case Study 1 illustrates the potential use of gene drives to prevent mosquitoes from transmitting dengue, a virus that occurs predominately in urban environments throughout the tropics. Dengue can also occur in rural and temperate zones, typically due to introduction by travelers from dengue-endemic areas. Dengue remains a major source of human morbidity worldwide, with more than 50 million cases occurring annually and 2.5 billion people at high risk of getting the disease (WHO, 2009). Another estimate places the burden at 390 million infections per year with 96 million clinical manifestations (Bhatt, 2013). More than 70 percent of people who are at higher risk of dengue infection (around 1.8 billion people) live in Southeast Asia and the Western Pacific region (WHO, 2009).

There are currently no curative treatments for dengue. However, in April 2016, the first ever dengue vaccine, Dengvaxia (CYD-TDV) by Sanofi Pasteur, was approved by the World Health Organization for use in endemic countries. Strategies using *Wolbachia* infected *Aedes aegypti* mosquitoes to reduce their populations or cause refractoriness to dengue infection are being evaluated (Dobson et.al 2002; Joubert et al., 2016); however, to date, the prevention of dengue has relied on ultra-low volume spraying of insecticides and removal of *Aedes aegypti* breeding sites, which are typically human-made containers. These strategies are laborious and typically reactive rather than proactive (Achee et al., 2015). Resistance to insecticides among targeted species is also challenging the efficacy of currently available chemicals. In ad-

dition, because dengue disease alternates between high and low incidences depending on the year and season, and because the potential serotype interaction and co-circulations, predicting and therefore preventing possible dengue epidemics is extremely complex. Given these challenges, a gene drive could, theoretically, provide enhanced sustainability for disease prevention, because repeated mosquito releases may not be required. A gene drive that suppresses the mosquito population might also provide a broader health benefit to human populations, since *Aedes aegypti* also serves as a vector for a range of other viruses responsible for human disease, including yellow fever, West Nile, chikungunya, zika, and eastern equine encephalitis. A suppression drive would also lead to a reduction in nuisance mosquito biting.

Case Study 2, on human malaria, describes a gene drive intended to prevent mosquitoes from transmitting the protozoan parasite that causes malaria, a major cause of human illness and death worldwide. Malaria occurs predominately throughout the tropics, but it can also occur in temperate zones, typically when travelers visit areas where malaria is present and bring the disease home with them. In 2013, 198 million cases of malaria were estimated to have occurred, leading to 584,000 deaths (WHO, 2014). Most of these cases occurred in Sub-Saharan Africa where the species of parasite responsible for severe disease, *Plamodium falciparum*, is most prevalent. Ninety percent of global malaria deaths occurred in Africa, with children under the age of five years accounting for 78 percent of deaths (WHO, 2014).

Human malaria infections can be cured using drug therapy, but therapy requires that the parasite be detected and that the infected person have access to health care. These requirements can be extremely challenging in many settings where malaria is endemic. In addition, the parasites have developed resistance to many first-line drugs. Insecticide treated bed nets, larval source management, and indoor residual spraying are strategies for preventing transmission, but they require organized campaigns and resources. Moreover, malaria carrying mosquitoes can develop resistance to the chemicals used in currently available Insecticide treated bed nets and indoor residual spraying programs, making control difficult. Malaria vaccines are under development and have shown promise, but will take many more years before they become fully effective, scalable for use and approved for wide application. The possible benefits of a gene drive that prevents mosquitoes from transmitting malaria would, in theory, include an impact on morbidity and mortality caused by disease, a reduction in nuisance mosquito biting experienced by inhabitants, and a sustainable approach to delivering an intervention within remote communities where resources may be limited and efforts for disease control most challenging.

Although these case studies are particularly prominent examples of how gene drives might be used to advance public health, a number of other, similar uses of gene drives have been envisioned. These include proposals to develop a gene drive to modify deer ticks so that they cannot transmit the bacterium *Borrelia burgdorferi,* which causes Lyme disease (Pennisi, 2015b), and a gene drive to eradicate the parasitic flatworms that cause Schistosomiasis (Esvelt, 2016). Other possible uses of gene drives to prevent infectious disease are likely to emerge. The news in 2016 that Zika may pose a surprising and exceptionally significant public health threat shows that potential uses of gene drives may have a very great sense of urgency. Given the fear prompted by such threats, it may sometimes be difficult to make a reasoned decision about whether a gene drive provides a good possible solution.

Potential Agricultural Benefits

Agricultural uses of gene drives are a second significant source of human benefit. For example, gene drives might turn out to be useful for controlling some weeds, a possible use explored in Case Study 6. As Palmer amaranth has developed resistance to glyphosate, it has become the most economically detrimental weed of cotton in the American South. The weeds compete with crop plants for water, light, and nutrients, resulting in lower yields. They can also become stuck in harvesting equipment, slowing production. The benefits of a gene drive that restored Palmer amaranth's susceptibility to glyphosate could include improved crop productivity and economic gains for farmers.

Agricultural uses of gene drives in low- and middle-income countries could have a significant impact on human welfare. If it were technically feasible, a gene drive that limited the germination of witchweed (genus *Striga*) could boost the production of rice, corn, millet, and other cereals in developing coun-

tries. Crop damage from *Striga*, a parasitic plant that penetrates the roots of the host plant and saps nutrients, is particularly extensive in Africa and Asia. In Africa, one species (*Striga hermonthica*) alone is responsible for $10 billion per year in crop losses (Pennisi, 2015a). Alternative solutions may be possible, including the development of witchweed-resistant crops, but the economic effect of witchweed remains extensive.

The Value of Science and Innovation

Because research into gene drives is still at a very early stage, a definitive account of the benefits they might generate is not yet possible. The benefits envisioned so far may not yet be adequately understood, and the technology might, as it develops, lead eventually to uses that cannot yet be foreseen. In discussing the technology's likely effects, it is therefore important to be cautious about any one way of articulating and framing its likely outcomes. In science, one line of research tends to lead to still other possible lines of research. The work that goes into developing one technology can present possibilities for yet other technological developments. This is part of the potential benefit of developing organismal models, such as the zebrafish (see Case Study 7) to study gene drives, and to explore their applicability to other vertebrates. The possibility that research will tend to foster further, as-yet-unknown scientific advances is itself a significant category of benefit.

The benefit of facilitating science raises some issues that are different from those of public health and agricultural applications. Like those applications, the benefit of basic science may be ultimately grounded in a belief that the work will lead to tangible improvements in public health, agriculture, or other areas. But the benefit would be indirect, open-ended, and hypothetical.

Additionally, the capacity of research on gene drives to foster advances in science and technology might also be considered valuable for a more immediate and less tangible reason. It may be rooted, to some degree, in an intrinsic value sometimes given to knowledge, understanding, and innovation. To possess knowledge is to have a belief that is not only true but justified by evidence and reason. To gain understanding is to develop an overall picture of the thing one understands, putting different pieces of knowledge together and critically reflecting on their relationship to each other. Innovation is valuable in good part, of course, because it often leads to economic benefits, but it may also be valued in itself: innovation puts understanding to work in the world in ways that may reflect creativity, diligence, planning, and leadership. Knowledge, understanding, and innovation therefore require and display capacities and virtues that are sometimes considered to make humans special, and they may also give one a special power in relation to the world. Finding intrinsic value in knowledge is also very much part of the tradition of science: Although this view of the value of science often goes unspoken, its significance is readily apparent (Sarewitz, 1996). It is probably the chief argument in support of sending probes to distant parts of our solar system and searching the galaxy for other solar systems. In biology, too, value is often attached to relatively arcane investigations that are unlikely to have an immediate impact on human welfare—such as trying to learn how life formed, how different living things came to be, and how long-extinct living things once lived.

The value that many people find in knowledge, understanding, and innovation is not always an overriding consideration in deciding whether to conduct research. That value may be outweighed by concerns about potential harms. However, it is a significant consideration, both in private life and in public decision making. From the standpoint of a scientist who decides to pursue the work described in Case Study 7, at least part of the rationale is likely to be a belief that it is intrinsically worthwhile. If the risks of research are minimal, then the perceived intrinsic value of the research, together with the possibility that it will lead to as-yet-unanticipated benefits, is likely to provide a very strong rationale for proceeding with basic research.

POTENTIAL HUMAN HARMS OF GENE DRIVES

Many of the possible harmful effects of gene drives have to do with environmental outcomes, which are considered in the next section. However, some gene drives pose potential harms to human well-being

if they do not function in field release as expected. Additionally, human harms might result from accidents in the laboratory (concerns about biosafety) or from any potential that gene drive research might have for deliberate misuse (concerns about biosecurity).

The release of gene-drive modified organisms has the potential to generate public health harms. One theoretical example is a mosquito modified so that it could not host the dengue virus that becomes a more susceptible host to another existing or new virus that harms human health. Another hypothetical outcome of this scenario is that the dengue virus might evolve a new phenotype that poses a slightly different hazard from the one that the gene drive was meant to suppress. A gene drive that suppressed rather than modified the host organism might have other effects. The removal of an entire species, such as a mosquito, could have effects on other organisms in the ecosystem, which could in turn lead to unwanted changes, such as an increase in the population of another insect disease vector as it fills the ecological niche opened by suppression of mosquito populations.

Gene drives developed for agricultural purposes could also have adverse effects on human well-being. Transfer of a suppression drive to a non-target wild species could have both adverse environmental outcomes and harmful effects on vegetable crops, for example. Palmer amaranth in Case Study 6 is a damaging weed in the United States, but related *Amaranthus* species are cultivated for food in in Mexico, South America, India, and China.

Deciding whether to go forward with a field release of a gene-drive modified organism will require a reasonable level of assurance that the possible harms have been identified and studied and that they are outweighed by the potential benefits, where the characterization of the potential outcomes involves both their significance (or severity) and their likelihood. The likelihood may depend not only on technical aspects of the gene drive and how it is expected to function within the organism, but also on environmental and societal issues. A positive balance of potential benefits over potential harms might mean that the harms are not very severe, that their likelihood of occurring is tolerable, that a reliable mitigation strategy can address potential harms, or perhaps that the potential harms are non-negligible but are still outweighed by the possible benefits. There are also trade-offs to consider (Finkel, 2011): The potential outcomes of a release will need to be weighed against the potential outcomes of alternative solutions to the problem for which the release is proposed, and also against the outcomes of doing nothing--which could amount to very great harm given an enormous, immediate, and highly certain public health problem. A gene-drive modified organism may offer a technological way of addressing a problem that was initially generated by larger societal and environmental problems, and if the technological solution provides a way of avoiding the larger issues, it may have the effect of perpetuating them. On the other hand, if the immediate problem is very serious, then a comparatively quick, targeted solution to it might be attractive anyway. Identifying the potential harms of a proposed field release will require case-by-case analysis and include use of a structured, systematic, and reasoned methods to investigate and model the possible outcomes, making use of everything known about the relevant species and ecosystems. Cost-benefit analysis may also be useful for modelling the possible outcomes of regulatory or policy decisions about gene drive research and use.

Although structured decision making tools for examining and modelling outcomes can provide useful guidance, they may not always be decisive given the questions of value on which they depend. While the outcomes might be tangible human interests, identifying them, articulating their significance, and determining the tolerable level of uncertainty about them are matters of value and may remain contested. The probabilities assigned to outcomes may also leave some uncertainty about how a proposed release will go. Moreover, some theoretical harms—such as the possibility that a pathogen might adapt to a gene drive and produce a new and worse phenotype—are hard to predict. How much certainty is needed in order to declare that the outcomes have been adequately studied is a further question of value. Resolving uncertainty takes time, and prolonging the analysis can sometimes prolong the problem. A society might opt for a more or less precautionary position with respect to uncertainty, declaring either that the uncertainties must be minimized as much as possible or that some uncertainty is acceptable when there are significant potential benefits (Kaebnick et al., 2014).

Some of the outcomes about which people may express concerns may be scientifically implausible. This can be a result of the complex ways in which technical information is generated and communicated in a society, particularly when it is connected to difficult value questions, and because of challenges of perception that are associated with some kinds of risks. Some kinds of potential harms are likely to be seen as more alarming than others for reasons that are independent of the degree or likelihood of damage (Slovic, 1987). Structured decision making tools may not assess outcomes in a way that is satisfying to those who are particularly alarmed by those outcomes.

The possibility that public attitudes about harms may seem irrational at times does not mean that public attitudes can be set aside. Both humility and prudence require deference to the public perceptions and understanding of research. Since benefits and harms are matters of value, it is impossible to say exactly which outcomes should be considered benefits, which outcomes should be considered harms, and how much weight they should be given without incorporating the publics' own views. Different publics may identify and gauge relative benefits and harms somewhat differently. Some members of the public believe that scientists irrationally over-estimate their ability to produce the benefits they propose. There is likely to be broad agreement that eliminating malaria and dengue would be good, but there might be differences of opinion about how that benefit compares against potential harms of gene-drive modified organisms, either to humans or to the environment. Moreover, a society that is affected by a disease may place a much greater value on eliminating that disease than would a society where the disease does not occur. There could also be reasonable differences of opinion about how much confidence we need in predictions about outcomes in order to decide whether to pursue a potential benefit (and incur some potential harms), or to take precautionary measures against the potential harms (and constrain progress toward the benefit). Issues of risk assessment, risk, perception, public engagement, and precaution are addressed further later in this chapter, as well as in subsequent chapters.

Dual Use Concerns

Research that might be put to deliberately malicious uses is sometimes known as dual use research (NSABB, 2007). The dual use potential of gene drives is not the same as that of other lines of research in synthetic biology. In principle, synthetic biology techniques can be used to synthesize pathogens or modify them in ways that make them more dangerous, and gain-of-function research on influenza viruses and other pathogens can be used not only to learn how to defend against those pathogens but also to create more potent ones (Presidential Commission, 2010). Gene-drive technologies would be inapplicable to bacteria and viruses (because they are limited to organisms that reproduce sexually), would not be effective on humans (because of humans' long generation times), and might be of limited effect on crops and livestock (because their reproduction is sometimes controlled in ways that would hinder propagation of a gene drive). Dual use potential is not necessarily a reason not to pursue the research. One common argument for pursuing research into the synthesis or modification of pathogens is that the best defense against dual use is a good offense: the research provides a basis for defending against those pathogens (Fauci et al., 2011). Dual use concerns about gene drives are also discussed in Chapter 8.

VALUES RELEVANT TO POTENTIAL ENVIRONMENTAL EFFECTS

There is a widespread sense among researchers and commentators that the capacity of gene drives to genetically alter a wild population, and potentially an entire species, represents a new type of ethical environmental challenge (Esvelt et al., 2014; Charo and Greely, 2015; Caplan et al., 2015). There are significant potential environmental benefits but also legitimate questions about potential environmental harms. The values attached to the potential environmental outcomes may be understood in different ways, some of which are not universally accepted. As a result, how they are to be weighed against each other and alongside public health and agricultural outcomes is very complicated.

Potential Environmental Benefits

Applications of genetic technologies in agriculture can lead to the accidental alteration of wild populations (Ellstrand et al., 2013, Lai et al. 2012). To date, no agricultural application has incorporated a mechanism specifically designed to force a change through a population as would a gene drive. The closest analog to what gene-drive technologies can accomplish in the shared environment is the use of genetic engineering to confer beneficial traits to threatened species, with the hope that, if genetically altered organisms were released in the environment, the engineered traits would drive through the population under the "natural" pressure of evolution. This kind of application is known as "facilitated adaptation." One example of facilitated adaptation is the effort now under way to impart resistance to chestnut blight to the threatened American chestnut through the transferring of genes from wheat, grape, Asian chestnuts, and other organisms (Newhouse et al., 2014).

Case Study 3, which describes a gene drive to prevent mosquitoes from transmitting avian malaria, highlights considerations for conserving threatened or endangered species. Avian malaria occurs throughout the world and on almost every continent, impacting several hundred species of birds. Parasites of the genus *Plasmodium* are responsible for pathogenicity, mass mortality, population declines, and even extinctions of many bird species (van Riper et al., 1986; Valkiūnas, 1993). In Hawaii, the fossil record shows that many events in the past have affected the size and diversity of populations of native birds. Hawaii's native birds live in a fragile habitat where any disturbance, from human settlement and hunting to diseases, leads to a drastic reduction of the species diversity. Avian malaria, caused by *Plasmodium relictum* and transmitted by *Culex quinquefasciatus* mosquitoes, is widely recognized as the greatest current threat to the Hawaiian avifauna, especially honeycreepers (Warner, 1968; Freed, 1999; van Riper and Scott, 2001). A wave of extinctions of native birds during the 1920s and 1930s has been attributed to avian malaria, and today native birds living at elevations below 1,500 meters continue to be at risk from malaria (Goff and van Riper, 1981; van Riper et al., 1980). In contrast, malaria has minimal impact on the survival of non-native birds, and because mosquitoes are rate at altitudes above 1,500 meters, higher elevations are theorized to be protective to native forest birds (Samuel et al., 2015). If a gene drive were developed either to reduce populations of the mosquito vector, or to make them refractory to infection with the malaria parasite, the susceptible birds might begin to repopulate the higher altitudes and reintroduce themselves into original ecosystems of lower elevations.

Aiding the threatened honeycreeper species through introduction of a gene-drive modified mosquito, for example, could potentially prevent the bird's extinction; however, such an intervention could also be expected to have unintentional impacts on the ecosystem as well as on the human population. For example, since the honeycreepers are nectar-feeders, there may be shifts in plant species biodiversity if the bird population is reintroduced into areas where they are currently not found. Competition with other birds for similar nesting and feeding sites could also occur, thereby modifying the diversity of other fauna.

Similar environmental benefits are at play in Case Study 4, which describes gene drives to suppress non-native rodent populations on remote islands such as are found in the Pacific. Mice and rats have been inadvertently introduced to these islands by maritime travelers with frequently catastrophic effects on native species and ecosystems. These effects are sometimes a result of direct predation by the rodents on the various native species, but they may also result from habitat alteration, competition for food, and other ecosystem interference.

A gene drive to control nonindigenous rodents is attractive in part because of the many challenges to control them using alternative methods. Initial efforts at population control involved the use of rodenticides, usually anti-coagulants. First-generation compounds, such as warfarin, had to be administered in high concentrations over multiple doses, they have now been replaced by second-generation compounds such as the odorless and tasteless toxicant Brodifacoum (Mensching and Volmer, 2008). The cost of administering these compounds is estimated to be in the millions of dollars due to expenses associated with their regulation, dispersal method, and actual inherent cost of the toxicant (Meerburg et al., 2008; Williams, 2013). Rodents can sometimes evade the chemicals. Moreover, the chemicals can result in a com-

paratively painful death for the affected rodents (Gould, 2015) and they may adversely affect the health of humans, other animals, and the overall ecosystem (Lorvelec and Pascal, 2005; Witmer et al., 2011).

Mechanical control methods, such as trapping, are not considered suitable to eradicate a rodent population, although they can be useful in conjunction with other methods. Two types of traps currently exist and are categorized based on the outcome to the rodent (Hygnstrom and Virchow, 1992; Witmer and Jojola, 2006). Kill traps such as snap traps are effective only on a small scale, while the effectiveness of glue traps and snares is questionable given the animal's ability to avoid them (e.g., jumping over them) (Witmer and Jojola, 2006). Kill traps also call into question the welfare of the animal and whether this method is in fact humane. Live traps are a non-lethal, arguably more humane, but expensive alternative to kill traps. While live traps tend to be successful for capturing rodents, the trapped rodents must then be relocated, which poses a further set of problems (Hygnstrom and Virchow, 1991; Witmer and Jojola, 2006). Collectively, these mechanical methods cannot discriminate between target and nontarget organisms (Lorvelec and Pascal, 2005), and so their use raises similar issues to that of chemical toxicants. In addition, traps require considerable human labor and monitoring, and may cause injury to the workers who place them. Finally, animals are able to adapt to these traps, which can be damaged easily by people or animals (Witmer et al., 2011).

Biological controls of invasive rodents include predators, parasites, and other disease-causing agents that act to limit the population. One of the considerations in using this type of method is whether the introduced organism would itself become invasive following its placement in an environment to which it is not endemic. Several unsuccessful applications of this method have taken place in the past. The introduction of rabbits into Australia in the late 1800s (Garden, 2005) required subsequent efforts to control their substantive, unexpected, population growth (Fenner, 1983; Saunders et al., 2010). The introduction of the cane toad to control agricultural pests of Australian sugar cane (Weber, 2010) had a similar, unexpectedly complicated outcome. The cost of this type of intervention will vary depending upon the targeted organism of interest and the biological control agent being introduced.

Other methods currently being explored to control non-native rodent populations take advantage of the process of RNA interference (RNAi), in which double-stranded RNAs might be delivered to the rodent to silence the expression of genes essential for life (Gao and Zhang, 2007). Technical issues associated with this technique include actual delivery of double-stranded RNAs, their inherent stability and thus persistence of inhibition, the concentration required to eradicate a species, their mechanism of spread, and their potential biosafety risks. Proof-of-concept, however, has been demonstrated with sea lampreys (Heath et al., 2014). Another possible method is the induction of autoimmune infertility, achieved through the introduction of a virus expressing proteins that elicit an immune response, and therefore target the fertilization process and prevent formation of the zygote (Chambers et al., 1999). This technique would reduce the target population, but challenges would remain with respect to the administration of the virus at the appropriate time in the rodent's life-cycle and the numbers of rodents to be infected (Jacob et al., 2008). It would also be necessary to ensure that infected rodents mate with one another as opposed to untreated rodents (Biotechnology Australia, 2001). Finally, in some instances it may not be possible to eradicate an invasive rodent population because doing so is cost-prohibitive, because of the location and topography of the land limit access, because the presence of humans would damage the ecosystem, or because of others harms posed to the area.

In short, there are many ways to try to rid an island of a nonindigenous rodent population and many reasons those methods are likely to fail (Gould, 2015). A gene drive that successfully affected the entire population may then appear particularly attractive. A gene-drive modified rodent could be released on an affected island with relatively little other human labor required, and perhaps at relatively low cost.

Potential Environmental Harms

The potential environmental release of gene-drive modified organisms will raise questions about possible harmful environmental outcomes. Case Studies 1 and 2, for example, the potential consequences for other species of reducing the mosquito population may need to be considered, especially given the

large geospatial scale at which the gene drive would likely be implemented. Some highly valued species may depend on the mosquito population, even in places where the targeted mosquitoes are nonindigenous. As previously noted, a gene drive to modified or eliminate Palmer amaranth in the American South, considered in Case Study 6, could affect closely related to some wild species as well as to food crops in other parts of the world. Spotted knapweed, the target of a gene drive considered in Case Study 5, is pollinated by insects, including butterflies; so as a result, there may be unintended environmental consequences that would require further before such a gene drive is pursued.

Restoring a bird species as in Case Study 6, may also have unexpected environmental consequences that need to be considered. An ecosystem can sometimes adapt to human alterations in ways that cannot be reversed without bringing about still more unwanted changes.

Using gene-drive modified organisms to bring about environmental changes is analogous in some respects to the past attempts to use biological controls to fight pests. As the history of unfortunate experiences with biological controls suggests, adequate assessment of the environmental harms of a proposed release will require careful, case by case analysis. Structured assessment tools for carrying out this analysis are discussed at length in chapter 5. One example of complex considerations that must be examined is whether the invading species plays a critical role in the ecosystem. For example, *Tamarix* (salt cedar) species have overtaken many riparian communities in the American Southwest, often as hybrids that are not found in their native ranges (Schaal et al., 2003). In the process, *Tamarix* has displaced native plants as the breeding habitat for approximately 50 native bird species (Sogge et al., 2008); and hence its suppression of this invasive species could have unintended consequences for native birds. Remarkably, *Tamarix* also alters the salinity of soil, which negatively affects the ability of native plants to re-colonize (Zavaleta et al., 2001), so sites must be restored prior to reintroduction of native species. Assuming that the technological obstacles of transformation and targeting could be overcome, gene drives to suppress *Tamarix* populations would likely spread slowly, because they are long-lived perennials, commonly spread vegetatively as well as sexually, and may have substantial population substructure, as is typical of asexually spreading organisms (Sakai et al., 2001). *Tamarix* nonetheless illustrates a long-standing complication: the eradication of an invasive plant species may lead to unexpected consequences, such as the loss of habitat for native species or even the establishment of a second, more resilient invasive species (Zavaleta et al., 2001).

Adequately assessing the environmental harms of a proposed release of a gene-drive modified organism also requires extensive engagement with those who might be affected by the release. As with the potential benefits, the harms cannot be adequately identified and weighed without that input. If the release is contemplated for a low- or middle-income nation, it is very important that people in developed countries avoid imposing their own views about what the benefits and harms are and how they should be weighed.

Intrinsic and Anthropocentric Values

Similarly, the public must be engaged in order to identify and weigh relevant environmental outcomes appropriately. In the applications described in Case Studies 3 and 4, for example, it would be important for researchers and project organizers to ask exactly why and in what way it is a benefit to rid an island of avian malaria or nonindigenous rodents and thereby try restoring a native population. Similarly, it is important to think about how the environmental harms should be understood. Different people may understand and value environmental outcomes in very different ways. Some people evaluate environmental outcomes in terms of human outcomes: An environmental harm is an environmental effect that has negative repercussions for human health and welfare, and an environmental benefit is an outcome that fosters desirable human outcomes. This way of thinking about environmental outcomes is at work when people speak of "ecosystem services," for example. Ecosystems perform a wide variety of functions that are vital to humans, communities, and societies, ranging from generating food to cleaning water to providing opportunities for recreation.

On the other hand, some people evaluate environmental outcomes not only in terms of outcomes for humans but also in terms of their effects on the environment itself—for example, the effects on biodiversity or on the richness and resilience of ecosystems, aside from ways in which biodiversity and ecosystem resilience are beneficial to people. This way of thinking about environmental outcomes is often at work when people express concern about endangered species. For example, although endangered species are sometimes valued for their ecosystem services, or for their economic or medical usefulness, they may also be considered valuable in and of themselves, because they are part of the shared environment. To see environmental outcomes as valuable in and of themselves is to think of naturally occurring environmental phenomena as intrinsically valuable and to adopt a preservationist stance toward those phenomena. Views about the intrinsic value of the natural world probably also play a role in efforts to protect "wild" places, such as through the U.S. Wilderness Act, the Wild and Scenic Rivers Act, and the national park system and other federal and state preserves, and such views may also have some role in the efforts to pass the U.S. Clean Air and Clean Water Acts.

Gene drives' unique mode of altering the shared environment poses special challenges, and perhaps also special opportunities, for those who take a preservationist stance toward the natural world. Genetic engineering techniques in general are sometimes perceived as intrinsically unnatural (President's Commission, 1982; Nuffield Council on Bioethics, 2015). Aside from whether the gene drive itself is perceived as unnatural, gene drives could have significant effects on particular organisms and ecosystems, such that the perceived naturalness of those phenomena, and of the places where they are found, could be substantially changed. More broadly, gene-drive technologies raise special questions shared by many environmentalists (although not all) about the ever greater powers that humans are developing to alter the natural world. From this perspective, gene-drive technologies might be seen as shifting the balance of power in significant new ways insofar as they may let humans overrule some "natural laws," such as Mendelian rules of inheritance and Darwinian conceptions of survival of the fittest. They may appear, to some people, to reflect the same human hubris, the same overeagerness to control nature and the same overconfidence that we could succeed at it, that have created many environmental problems. In the case studies considered above, the clearest human benefits have to do with such human needs as avoiding disease and providing food, but perhaps, at some point in the future, gene drives could be developed in which the benefits involve human preferences and fancies. Perhaps gene drives could be used to suppress or modify populations of insects merely on the grounds that they are nuisances, for example. Following the news in 2016 that Zika virus, transmitted by the mosquito *Aedes aegypti*, might present a significant public health threat, some discussion appeared in the popular media about whether mosquitoes in general should be eliminated—those that are annoying as well as those that pose public health threats. In principle, some might also propose to use gene drives to make wild species more aesthetically pleasing. Zebrafish genetically engineered to be fluorescent are now sold as pets, and kits are available on the Internet that allow customers to produce mustard plants engineered to glow faintly in the dark.[1] In theory, gene drives could allow individuals to propagate such traits in wild populations.

Questions about how to define "nature" and how to understand the value attached to nature raise a number of difficult philosophical and social problems (Cronon, 1995; Soper, 1995; Sagoff, 2003; Thompson, 2003; Marris, 2013; Kaebnick, 2014; Nuffield Council on Bioethics, 2015). Skeptics of concerns about nature argue that no entirely natural phenomena exist any longer, for example, and that human intervention into nature is already common and sometimes (in medicine, for example) widely accepted. In the long-running debates about genetically engineered crops and livestock and about the use of genetic technologies to treat or perhaps even to enhance human beings, skeptics have also argued that concerns about nature are based on religious, superstitious, or personal psychological reactions that are not easily defended in the kind of public discourse that should support public policy making. Similarly, skepticism about "nature" might itself reflect corporate and other interests in the activities and technologies that are sometimes seen as unattractive alterations of nature.

[1]Experience the Glo!, https://www.glofish.com/; Natural lighting without electricity, http://www.glowingplant.com/.

These debates about nature will continue, and gene-drive modified organisms may be a significant new moment in them. In a survey of the use of new genetic technologies on non-human organisms, bioethicists Alta Charo and Henry Greely have observed, for example, that some people "decry the 'end of nature' and the loss of the sense of a reality outside ourselves, whether created by God or by nature, [and] feel impoverished by the increasing human footprint on the world…Even those not reflexively against 'unnatural' changes through biotechnology might find something unsettling about altering the biosphere with uses that are recreational, whimsical, or even Disneyfied" (Charo and Greely, 2015). On the other hand, those who resist genetic engineering because they see it as "unnatural" have to confront the possibility that gene drives might sometimes be very valuable tools for conservation, as illustrated in Case studies 3, 4, and 5 (Jennings, 2015; Webber et al., 2015).

The intrinsic value that many find in the natural world presents an interesting comparison to the value that many find in knowledge, understanding, invention, innovation, and industry. In some ways, these two stances may be similar; like the value found in knowledge, understanding, and innovation, concerns related to the intrinsic value of nature, and how to compare those concerns to more tangible human benefits and harms, will be contested in debates overpublic policy. The two kinds of value also contrast with each other to some degree; finding value in nature seems to call for adjusting human activity in order to accommodate nature, while finding value in knowledge, understanding, invention, innovation, and industry seems to celebrate the alteration of nature to support human activity. On the other hand, it may be possible for an individual, community, or society to share both values to some extent. Perhaps, each stance even implicates the other: Preservation of natural phenomena can be aided by appropriately directed efforts to understand and intervene in the world, and human activity in the world depends on trying to accommodate the natural world.

This report does not side with any particular way of understanding these issues and does not resolve them. They are left here as open questions, and are part of a growing and heated debate among environmentalists about the values that underpin environmentalism. Historically, in the United States, some environmentalists have leaned toward preservationism, tracing their thinking back through Aldo Leopold's "land ethic" to John Muir's call to protect Yosemite and Henry David Thoreau's celebration of wildness and of places that exhibit untrammeled wildness and limited human impact. Others have leaned toward thinking of natural phenomena in terms of ecosystems services—a stance that is often called conservationist and traces back to Gifford Pinchot and the creation of the U.S. Forest Service (Rich, 2016). Recently, some environmentalists have proposed that these two sides could be and should be bridged with a third, middling position, perhaps a "gardening ethic" that values alteration of nature and accommodation of nature simultaneously (Pollan,1991; Marris, 2013; Rich, 2016). The evolving debate about the desirable human relationship to nature is also reflected in the idea that the earth has entered the Anthropocene, defined as an epoch in which human influence in nature will leave a geologic record (Waters et al., 2016). Passing this boundary is seen sometimes as evidence of the need for greater restraint toward nature, and sometimes as showing that humans should accept a strongly interventionist role in nature, for they are in that role whether they like it or not. However these questions about the value of nature and the proper human relationship to nature are understood, they are likely to be very important in the public's response to gene-drive technologies and in decisions about how those technologies should be developed and used, given the prospect that gene drives could be a tool for modifying wild species to suit human needs, perhaps to bring about their extinction, perhaps to alter them to suit aesthetic preferences. Moreover, different publics will undoubtedly frame these questions differently. The views about nature that have been described here are found predominantly in Western cultures, and probably particularly in the United States, since European views of "nature" are more likely than American views to see natural phenomena as part of agricultural contexts—and to see agricultural phenomena as part of "the shared environment" (Soper, 1995).

CONCERNS ABOUT JUSTICE

In addition to questions about various kinds of potential benefits and harms, research on gene drives presents questions of justice. Questions of justice differ from questions about potential benefits and harms in that they are more about *who* than *what*: They are about who would be affected by the benefits and harms, who will be able to conduct research into gene drive technologies and study the release of gene-drive modified organisms, and who will make the decisions about whether to pursue the benefits and risk the potential harms. They are questions about the distribution of potential benefits and harms, about liberty, about the nature of legitimate decision making for matters affecting the public. They are about how communities and nations are affected by gene-drive technologies, the ability of scientists and funders to undertake the research, and the relationship of citizens to nations and of nations to each other.

Some of the envisioned uses of gene drives are motivated in large part by concerns about justice. Part of the value of Case Study 2, for example, is that the people who are most seriously affected by malaria are in low income countries whose health (and other) needs have often been overlooked by wealthier, more developed countries. Cures for malaria have been available for a long time, but they are seldom available to the people who need them most. The most at-risk countries, where malaria is a very significant burden for communities and governments, often have limited health care systems and little capacity to fund or conduct medical research. In sub-Saharan Africa, where the burden is greatest, diagnosis and treatment alone, excluding prevention strategies, are estimated to have cost about $300 million per year since 2000 (WHO, 2014).

In several of the case studies, concern about the distribution of benefits, set against the history of the relationships between high-income countries and lower-income countries, is part of the reason to move forward with the research. However, concerns about justice can also present reasons to be particularly cautious about a gene-drive modified organism. In Case Study 6, the gene-drive modified Palmer amaranth envisioned to suppress the population might be beneficial in the United States, where Palmer amaranth is a pest, but be harmful if it were to make its way to Mexico, South America, India, and China, where related *Amaranthus* species are cultivated for food. In such a case, a comparison of the benefits to the harms involves not only an understanding of their magnitude and likelihood, but also of the relative life circumstances of the people who would experience them and perhaps even of the histories and relationships of the countries in which those people live. Similarly, some societies could be understandably cautious and give researchers little latitude to proceed considering release of a gene-drive modified organism that has been developed by researchers from high-income countries, that would be proposed for release in a low-income country, and whose benefits and harms cannot be fully known in advance of the release. For Case studies 1 and 2, any harms from the release of gene-drive modified mosquitoes are likely to be borne disproportionately by low- and middle-income countries. If the research in those cases is driven by researchers and funders from wealthy countries, researchers and other decision makers may tend to underestimate or discount the risks. On the other hand, as noted earlier, the people who are immediately affected by a disease are the most likely to understand its true burden; those from wealthy countries may tend to discount the benefits that others value.

These questions about disproportionately distributed benefits and burdens highlight the importance of the relationship between researchers and funders from wealthy nations and those in poorer countries who must live with the consequences of research in their environs. If an environmental release of gene-drive modified organisms leads to unanticipated public health or environmental harms and for which no mitigation strategy has been put in place, the researchers and funders bear a responsibility not to abandon the people enduring those harms. Withdrawing from the community can give rise to feelings of abandonment and a sense of loss (Lavery et al., 2008). In short, a strong and long-term relationship between communities and researchers is deeply important (Brown et al., 2014; King et al., 2014).

Another set of concerns about justice centers on who is involved in decisions about the development and use of gene drives. People hold a wide variety of views about justice, especially if the scope of inquiry is not limited to Western democracies, and these different views could lead to different expectations about the roles of research in society and how research should be conducted. There may be a loose con-

sensus that benefits of research should not all accrue to the wealthy while all the harms are borne by people who are poor and powerless, but there is also some general agreement that scientists should have liberty to pursue their research as they do not cause harm to others. This loose consensus leaves room for meaningful disagreement, where different people could largely agree on the likely outcomes of releasing a gene-drive modified organism into the environment but still come to different conclusions about whether the release is a good idea.

In the absence of any strategy for resolving such questions, the best course of action is to ensure that the people who could be affected by a proposed project or policy have an opportunity to have a voice in decisions about it. Experts acting alone will not be able to identify or weigh the true costs and benefits of gene drives (Kaebnick et al., 2014; Sarewitz, 2015). In other words, justices require procedures that allow both broad public decision making about the development and use of gene drives and local community decision making about specific proposed releases of gene-drive modified organisms. The ability of people in low-income countries to participate meaningfully in decision making would be supported best not by merely engaging them in decision making but by building the capacity in those countries to conduct research that is locally valuable, regulate and provide oversight of gene drive research generally, and carry out their own decision making about its application. To ensure that capacity-building activities are not just a guise for off-loading expensive and risky research—perpetuating rather than addressing injustice—such activities need to include the development not just of technical capacity to do research but also of capacity to oversee safe and responsible research practices and decide how best to use research findings. Genuine capacity-building must be understood as empowerment, and empowerment must mean that a community or country is able to act on its values rather than merely relying on values imported from elsewhere.

Selecting Sites for Field Tests or Environmental Release of Gene-Drive Modified Organisms

A special issue that arises in research involving genetically modified organisms is the selection of sites for conducting confined field trials and perhaps for releasing the organism into the environment. A variety of research publications address site selection for release of mosquitoes that have been genetically modified in ways that do not involve gene drives (Brown et al., 2014; Lavery et al., 2008). Researchers working on gene-drive modified mosquitoes and other organisms should bear in mind the recommendations from these publications, not only for guidance on matters of justice, but also for practical guidance. Site selection should be guided by many considerations, including the balance of benefits and harms, both in terms of public health and the environment and as understood in collaboration with the stakeholders in the community (as discussed above); the feasibility of examining outcomes through structured tools such as risk assessment (as discussed in Chapter 5); the feasibility of community engagement (as discussed in Chapter 6); and appropriate governance structures within the host country (as discussed in Chapter 7). It is important to be able to establish a relationship with the community stakeholders (Brown et al., 2014; King et al., 2014), learn about the community's own understanding of its interests, establish trust, navigate the regulatory structure, and follow through on commitments made to the community (Brown et al., 2014; Lavery et al., 2008).

Environmental release of gene-drive modified organisms also raises issues that go beyond the selection of a specific location for the release. While some kinds of genetically modified mosquitoes are likely to disappear from the environment unless they are released repeatedly, gene drives are designed to propel a trait through an entire population, moving beyond any single community and crossing national boundaries as well. Deciding when and where to release a gene-drive modified organism requires attention to national, regional, and perhaps even global concerns in addition to the concerns of the local community.

Other Analyses of Gene Drives and the Issues They Raise

There is no well-developed public debate yet about gene drive research, as there is about genetically engineered organisms in agriculture. In the academic literature to date, only a few analyses have addressed at length the ethical issues raised by gene-drive modified organisms.

Commentators have been nearly unanimous that gene drive technologies might have very significant, tangible benefits in a variety of contexts, especially public health, agriculture, and environmental conservation, and they also agree that there are a variety of questions about the potential harms of gene drive technologies, both to humans and to the environment. Questions have been raised, for example, about whether engineered gene drives will have the intended effects on target organisms (Oye et al., 2014; Caplan et al., 2015), and, in particular, whether the transmission of disease might be worsened when the target organism is a vector (Core Working Group on Guidance for Contained Field Trials of Vector Mosquitoes Engineered to Contain a Gene Drive, 2008); whether gene drives might spread to other organisms (Oye et al., 2014); what effects gene-drive modified organisms might have on humans who consume them (Caplan et al., 2015); what effects they might have for other populations of organisms and for ecosystems (Webber et al., 2015; Oye et al., 2014; Caplan et al., 2015); and what dual use potential they might have (Gurwitz, 2014; Oye et al., 2014). These concerns are most significant for possible field releases of gene-drive modified organisms, but scientists engaged in gene drive research have also recognized the importance of ensuring that laboratory work is conducted safely (Akbari et al., 2015). These concerns have not yet led any scholarly commentators to call for a halt to research on gene drive technologies, but they have led to many recommendations that would constrain and guide such research.

Several Existing analyses address several broad themes. One concerns uncertainty: The outcomes of gene drives are, for the time being, highly uncertain because of unresolved questions about how a given gene drive will function (for example, whether there will be off-target or pleiotropic effects, the nature of potential gene-environment interactions, and whether the gene drive could create selective pressure for yet other undesirable effects), about whether the gene drive will be transmitted to other, unintended populations of similar or different organisms, and about the overall effects of engineered gene drive mechanisms on ecosystems and humans. Recognition of this uncertainty has led commentators to recommend that research and related applications proceed only if a number of precautionary measures are in place. Among the recommendations that have been advanced are that research should be made public, with concepts and intended applications published in advance of construction and testing (Oye et al., 2014); that risk assessment should be conducted on a case-by-case basis to examine the possible outcomes of any release (Core Working Group on Guidance for Contained Field Trials, 2008; Oye et al., 2014); that research on a possible environmental release should occur in stages, from laboratory through preliminary trials, with each stage providing opportunities for feeding data back into decision making (Core Working Group on Guidance for Contained Field Trials, 2008; Oye et al., 2014; Caplan et al., 2015); and that a drive should not be developed unless mitigation methods or so-called immunizing or reversal drives are also developed (Oye et al., 2014; Caplan et al., 2015). The constraints appropriate for gene drive research are discussed in Chapters 2, 5, and 6.

Such recommendations appear to endorse a moderate degree of precaution about gene-drive technologies, although the concept of precaution in scientific research is understood in various ways and is hotly contested. Often, precaution is understood as a single general principle. One widely cited formulation holds that, if preliminary scientific evidence suggests that a proposed activity poses "threats of harm to human health or the environment," then measures should be taken to forestall the possible harms, and the activity's proponent or proponents shoulder the burden of proof in establishing that the activity should proceed.[2] Some critics of synthetic biology have endorsed this formulation of precaution (FOE, 2012). Others argue that a precautionary principle could be specified in a variety of ways, giving different policy responses to the proposed action and identifying different conditions that would warrant the response (Parke and Bedau, 2004). Precautionary principles could therefore vary both in the stringency of the restraints they impose on an action and in the sensitivity of the trigger. Other commentators describe precaution not as a principle but as an "attitude" or approach that is characterized by asking that a stronger case be made for an activity, and more assurances provided about it, than in a "proactionary" approach to proposed activities (Wolf, 2014). In a

[2]Wingspread Statement on the Precautionary Principle. 1998. The precautionary principle. Available at http://www.sehn.org/state.html.

similar vein, the Presidential Commission for the Study of Bioethical Issues recommended that synthetic biology be approached with "prudent vigilance," which the commission saw as a middle-of-the-road position between a strong precautionary stance and a strong proactionary stance. In a discussion of research on genetically modified mosquitoes, El-Zahabi-Bekdash and Lavery (2010) conclude that the goals of a precautionary "mindset" can be achieved in part through community engagement, since the community may be able to provide critical insights about potential harms. Strong formulations of precaution have come under a variety of criticisms, most notably that precaution will lead to inaction (Sunstein, 2005); however, by specifying constraints that allow research to continue, the commentary to date on gene drives deflects such criticisms. Further details on ways to incorporate precautionary steps into the conduct of gene drive research are discussed in Chapter 4.

Structured tools for modeling outcomes play an important role in decision making about how to use gene-drive modified organisms. As noted above, risk assessment is important in considering proposed environmental releases, and cost-benefit analysis may be helpful for informing regulatory and public policy decisions. Public examination of the costs and benefits will be particularly important if the development and use of gene-drive modified organisms depends primarily on public or philanthropic funding. Using cost-benefit analyses in a way that can support anticipatory governance presents challenges. At an early stage in a technology's development, there may not adequate information available to compare the potential benefits and harms of using that technology or to compare those outcomes to other possible strategies for addressing a given problem. In addition, highly formal cost-benefit analysis, in which benefits and harms are estimated as sums of money, is criticized on grounds that it distorts or omits some of the public's values (MacLean, 1998; Mandel and Gathii, 2006; Kysar, 2010; Sinden, 2015). Any intrinsic value that is assigned to wild species or to the natural environment, for example, may not be easily monetized.

The existing scholarly commentary is in agreement that gene drives might have broader environmental harms that need assessment, but the language used to express this concern varies. As discussed above, Charo and Greely consider that the environmental harms might in part reflect concerns about the extent of human impact over the natural world; indeed, what count as environmental benefits from a human perspective might nonetheless raise objections from some quarters (Charo and Greely, 2015). In examining the potential for gene drives to advance the conservation of ecosystems by eliminating invasive species Webber et al. (2015) express the underlying value as a question of national biosecurity that should be addressed by the countries where the species in question are found. Oye et al. (2014) argue that the effects of gene drives on genetic diversity warrant consideration, although they do not discuss whether genetic diversity is valued because it may produce human benefits or for its own sake. Caplan et al. (2015) ask whether using a gene drive to eliminate a species would "upset the ecological balance," which they suggest might override potential human benefits of the drive.

Perhaps precisely because the appropriate language for identifying, expressing, and weighing these value considerations is unclear, the scholarly commentary calls for public discussion of gene-drive technologies, and it holds that this discussion should occur both at a broad, societal level and at a local, community level corresponding to the site at which a gene-drive modified organism might be released. Public engagement is usually understood in these works not merely as a process of informing the public about gene-drive technologies, nor merely as a process of winning the public's acceptance, but as a process in which the public has meaningful opportunities to deliberate and contribute to decisions about whether and how to use gene-drive technologies. Public engagement therefore also provides an opportunity for public consideration and input as what constitutes beneficial and harmful outcomes, how to deal with uncertainty about those outcomes, what level of precaution to endorse, and how to understand the human relationship to nature. Public engagement is taken up in detail in Chapter 7. Public engagement in order to undertake a risk assessment is discussed in Chapters 6.

Engaging with members of the public is complicated by variations in the perception of risk. In risk assessment and in this report, risk is understood to involve measurable parameters—the statistical likelihood and the severity of a given harm. A considerable body of psychological research attests, however, that how people perceive and evaluate risks involves more than these measurable parameters. The risk of a harmful outcome is likely to be perceived as greater for some types of harm than for others (Slovic,

1987). Those risks of harm likely to be seen as greater are distinguished in psychometric research as being unfamiliar, uncontrollable, imposed rather than voluntarily accepted, associated with a sense of dread, and catastrophic (Slovic, 1987).

Genetic technologies rank high on these measures (Slovic, 1987). Gene drives might rank particularly high if their capacity to alter shared environments is associated with a marked sense of dread and unfamiliarity and if their capacity to be "invasive" is seen as a lack of controllability. The issues raised by attempts to release genetically engineered mosquitoes in the Florida Keys in order to drive down populations of dengue-transmitting mosquitos may illustrate the challenge confronting the use of gene-drive technologies (Alvarez, 2015). Public distrust of genetically engineered crops and livestock may encourage a similar distrust of gene drives. The fact that gene-drive modified organisms would be deliberately introduced into wild populations and comparatively less managed environments may cause some members of the public to see them as even more unattractive than other genetically modified organisms. On the other hand, gene drives systems might turn out to be less threatening than other genetic technologies if they can be put to significant conservation purposes and if they are not seen as reflecting corporate interests and a disregard for the environment. Such considerations show the importance of being wary about any one way of framing gene-drive technologies, and they also reveal some challenges to be addressed in public and community engagement.

Finally, the scholarly commentary raises questions existing governance. Oye et al. (2014) suggest that U.S. regulations may be inadequate for gene-drive modified organisms in general and may not apply to insects at all (see Chapter 8). Others have raised the question of whether U.S. regulations would apply to drives designed to be inserted into plants without using a plant disease vector (Caplan et al., 2015). Oye et al. (2014) also suggest that both U.S. and international security regulations may not apply to drives that raise dual use concerns because those regulations rely on lists of agents and may not include gene drives. Webber et al. (2015) hold that the decision of whether to use a gene-drive modified organism to try to eliminate an invasive species requires a regulatory framework that provides a mechanism for working through the relevant concerns. Governance of gene drive research is discussed at length in Chapter 8.

CONCLUSIONS

Questions about responsible science and applications of gene-drive technology rest on values at every step, from why and how research should be conducted to whether and where a gene-drive modified organism could be released into the environment. Values are also implicit in the development of appropriate governance for this new field.

Key value-based questions concern the determination of the potential benefits and harms of gene drives to humans and the environment. There are also questions about who would benefit, who would be harmed, and who would make decisions about gene-drive technologies. A third area concerns the place of humans in ecosystems and our larger relationship to nature. Some of these questions echo considerations in debates about genetic engineering.

Considerations regarding the potential benefits and harms of gene-drive modified organisms will be central in deciding whether to allow field testing or open environmental release. Understanding and comparing potential outcomes involves a number of challenges. Benefits and harms can be identified and assigned appropriate weight only case by case and only with the input of the people who will be affected by the release. Perceptions of outcomes may also be affected by a range of cultural and psychological factors in addition to the statistical likelihood and the quantifiable severity of a given harm.

Not everyone will be affected by gene drive research and applications in the same way. When selecting sites for field trials or open environmental releases, it will be important that researchers consider the values of the publics affected by the release and their understanding of the balance of benefits and harms. The expectation that people should have a voice in fundamental decisions that affect their health and their environment is particularly important and may generate additional guidelines for the release of gene-drive modified organisms. Approaches to ensure that communities participate meaningfully in deci-

sion making about the use of gene-drive modified organisms will be essential, particularly in low and middle-income countries where power differentials may affect such participation.

Perspectives on the place of human beings in ecosystems and our larger relationship to nature—including human impact on and manipulation of ecosystems—have an important role in the emerging debate about gene drives. The increased power that gene-drive technologies might give human beings to alter, and perhaps eliminate, wild species, thereby altering the shared environment, will be intrinsically objectionable to some people. An increased ability to conserve species and ecosystems or protect public health through gene-drive technologies may be intrinsically attractive to other people.

Developing public policies for gene-drive modified organisms will require careful attention to the human relationship to nature, a need that is amplified for proposals to use gene drives in ways that could lead to the extinction of species or significantly alter the environment.

Some of the fundamental reasons to conduct gene drive research include widely-shared commitments to fighting human disease, promoting human welfare, and protecting and restoring the natural environment. In addition, research on gene drives aligns with the intrinsic value that many people find in the pursuit of knowledge, understanding, and innovation. However, widely shared commitments to protect human welfare and the environment also provide reasons to develop public policy guidelines that may constrain research on gene drives or the releases of gene-drive modified organisms. Integrating precautionary measures into the research process can help to balance these potentially conflicting commitments—for example, by using structured tools to assess potential benefits and harms, by providing ample opportunities to gather further information about potential outcomes and revisit decisions about how to proceed, and by ensuring that people who will be affected by a proposed release are integrated into the decision making process.

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5

Phased Testing and Scientific Approaches to Reducing Potential Harms of Gene Drives

The acceleration of gene drive research and the increasing ease of use of the molecular technologies required to construct gene drives has generated considerable excitement about the potential use of gene-drive modified organisms to address public health, conservation, agricultural, and other challenges. However, releasing a gene-drive modified organism into the environment means that a complex molecular system will be introduced into complex ecological system, potentially setting off a cascade of population dynamics and evolutionary processes that could have numerous reverberating effects. Thus, effective strategies to carry out laboratory and field research are needed to study each type of gene-drive modified organism, its potential benefits and harms, and approaches to reduce or mitigate the potential harms.

The preceding chapters of this report describe what is known about gene drives, key population ecology and ecosystem considerations for gene drive research and human values that may influence whether and how gene-drive modified organisms are used. Building upon that foundation, this chapter focuses on a step-by-step pathway designed to guide research and support evidence-based decision making at each phase. In addition a range of strategies to reduce potential off-target and non-target effects are explored through the lens of this phased approach to research. Specific examples from biocontrol and existing transgenic research geared toward the suppression or modification of populations in the wild provide additional insights and lessons learned.

THE PHASED TESTING PATHWAY

Will proposed applications of gene drives work as intended? Researchers have proved that gene drives can be developed in some laboratory populations, but to date gene-drive modified organisms have not been studied in the environment. When will gene-drive modified organisms developed in the laboratory be ready for field-based research, or release into the environment? From a research perspective, the answer to these types of questions requires careful analysis of gene drives that begins at the molecular level and continues through the population and ecosystem levels. A number of criteria must be met for gene drives to be responsibly developed. A step-by-step approach can guide research from the laboratory to the field. To help guide gene drive research, the committee adapted and expanded upon the *phased testing pathway* outlined by the World Health Organization (WHO) for the testing of genetically modified mosquitoes (WHO/TDR, 2014).

A phased testing pathway is a step-wise approach to guide the preparation for and conduct of research that begins in the laboratory and continues through, if applicable, environmental monitoring (see Figure 5-1). The idealized pathway for research on a gene-drive modified organism includes five steps: Preparation for Research (phase 0), Laboratory-Based Research (phase 1), Field-Based Research (phase 2), Staged Environmental Release (phase 3), and Post-Release Surveillance (phase 4). Although the overall goal is for unidirectional movement from early to later phases, the pathway includes a set of feedback loops, to encourage repetition and refinement of studies based on new findings and data generated during the course of research. The phased testing pathway enables a researcher to identify milestones and decision points in regard to when the research is ready to move from one phase to the next. The decision to advance to the next phase of testing may also depend on approval from relevant publics, particularly local

communities and regulatory authorities. Hence, support mechanisms for risk assessment, public engagement, and governance, are needed throughout the phased testing pathway. Considerations for public engagement and governance are discussed in Chapters 7 and 8, respectively. Some examples of activities that take place in each phase are provided in Box 5-1 and discussed in the text below.

The goal of gene drive research is to develop organisms that are viable in the environment, and that, in some cases, will persist for indeterminate periods of time. In addition, gene-drive modified organisms may potentially be able to interbreed with related, wild species. For these reasons, confinement and containment are critical considerations throughout the phased testing pathway. In reviewing the literature on different approaches to research, the committee noted that the terms confinement and containment are often used interchangeably. To provide clarity, the committee developed the following definition derived from the 2004 National Research Council report, *Biological Confinement of Genetically Engineered Organisms*, the World Health Organization (WHO/TDR, 2014), and the U.S. Department of Agriculture:

- *Confinement* is the use of ecological conditions or biological methods to prevent unintended or uncontrolled persistence of an organism in the environment. Climatic isolation, when the surrounding environment or expected seasonal change is suboptimal for an organism's survival, is an example of ecological confinement (Akbari et al., 2015, Adelman, 2015a). An example of a biological method (sometimes called *bioconfinement*) is use of an organism that depends on a chemical or nutrient that is not present in the environment.
- *Containment* is the use of human-made or natural physical restrictions to prevent unintended or uncontrolled release of an organism into the environment. Examples of human-made containment mechanisms include large cages, greenhouses, and aquaculture pens (NRC, 2004). Geographic isolation, such as an island setting without human inhabitants (O'Connor et al., 2012) is an example of a natural physical barrier.

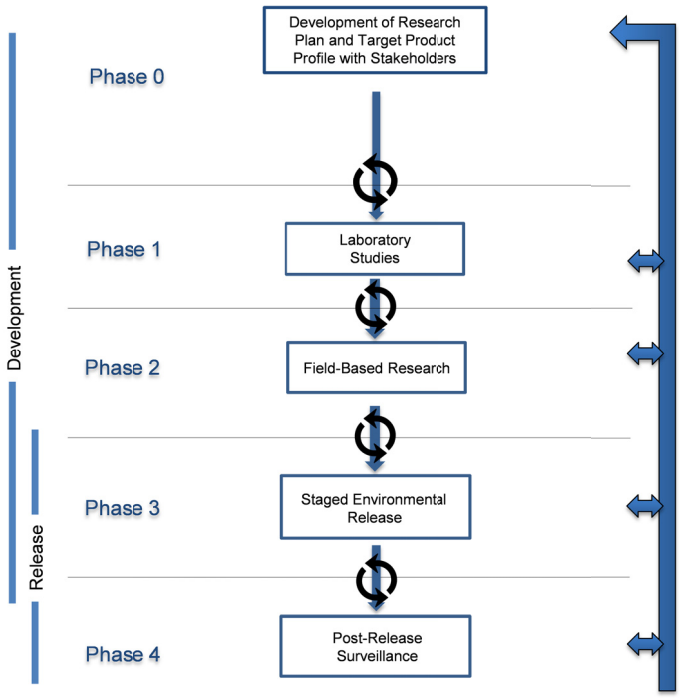

FIGURE 5-1 Phased testing pathway for gene drive research. The determination to advance from one phase to the next is based on set milestones and decision points. Pathway adapted from the World Health Organization's Guidance Framework for Testing of Genetically Modified Mosquitoes (WHO/TDR, 2014).[1]

[1]http://www.who.int/tdr/publications/year/2014/Guidance_framework_mosquitoes.pdf.

Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values

BOX 5-1 Example Activities to be Performed during Each Testing Phase

Phase 0: Research Preparation

- Develop a Target Product Profile
- Identify and plan for regulatory requirements
- Use models to inform standards, thresholds of acceptance, and study design
- Establish site-selection criteria (if research includes phase 2-4 trials)
- Identify risk assessment needs
- Identify appropriate confinement and containment strategies

Phase 1: Laboratory-Based Research

- Acquire required laboratory regulatory approvals
- Develop containment strategies
- Develop mitigation strategies
- Detect and measure off-target effects
- Optimize design of guide RNAs (when using CRISPR/Cas9-based gene drives)
- Utilize an optimized endonuclease with high cutting efficiency and accuracy
- Optimize for the use of homology-directed repair versus non-homologous end joining in order to maximize precision of editing
- Evaluate effects on organismal fitness in the presence of the gene drive
- Evaluate gene drive stability over multiple generations
- Mark gene drive organisms
- Use quantitative and computational methods
- Set baseline population-level effects

Phase 2: Field-Based Research

- Acquire site-specific regulatory approvals
- Validate efficacy
- Validate population-level effects
- Estimate impact on selected non-targets

Phase 3: Staged Environmental Release

- Acquire site-specific regulatory approvals
- Conduct monitoring and surveillance for efficacy
- Conduct monitoring and surveillance for harms

Phase 4: Post-Release Surveillance

- Acquire regulatory approvals
- Conduct monitoring and surveillance
- Measure impact

A combination of confinement and containment methods will likely be needed for each phase of gene drive research, with careful consideration for combinations that will not conflict with the purpose of the study.

Phase 0: Research Preparation

The purpose of the research preparation phase is to develop a robust plan that details the scope and goal of the study, pre-defined thresholds for success, methods of confinement and containment, and strategies to reduce the potential for unintended harms. Such a research plan can serve as the basis for funding proposals. At this stage, researchers have a working knowledge of the biology, behavior, and natural history of their target organism, as well as the environment or environments (e.g. laboratory or field contexts) in which the research will take place. Confinement, containment and biosafety mechanisms, mitiga-

tion strategies, and anticipated regulatory approvals will be developed and discussed with the relevant Institutional Biosafety Committees (IBCs), expert advisory panels, regulators and funders.

A critical component of this phase is a process for setting goals and pre-defined thresholds for success. A Target Product Profile (TPP), a strategic development process tool that uses set of criteria to pre-define ideal attributes of a candidate "product" (Food and Drug Administration, 2007), is one model. Although originally developed to facilitate assessment and prioritization of candidate pharmaceuticals, the TPP process has been adopted for the context of mosquito vector control product development by the WHO Vector Control and Advisory Group and by private funders such as the Bill & Melinda Gates Foundation.

A TPP can help researchers, funders, and policy makers to think through minimum standards of acceptance related to efficacy, safety, regulatory, and manufacturing endpoints for a specific application, such as the use of a gene-drive modified mouse to reduce the population of invasive wild mice on islands (Case Study 4) or the development of a gene-drive modified zebrafish as a vertebrate model for research on inheritability of off-target effects (Case Study 7). The TPP can also include specifications other than efficacy that will be important for policy decisions, such as cost comparisons of different potential courses of action, in order to weigh options and make sound decisions regarding the investment of finite resources. Table 5-1 shows a hypothetical TPP for a gene-drive modified organism.

Decisions about which specifications should be included in a TPP, including the standard endpoints to measure and minimum thresholds that should be met, are typically made by a range of stakeholders including academics, industry stakeholders, regulators, and policy makers. Some of these stakeholders can also be responsible for oversight and monitoring of research to ensure due diligence and compliance by researchers. The standards listed in the TPP are then incorporated into study designs and used to inform decisions regarding whether to move from one phase of research to the next. Another key component of phase 0 is establishing site selection criteria for proposed field-based research or staged releases of gene-drive modified organisms in the environment. The criteria are anticipated to be organism- and application-specific and reflect scientific goals, considerations for subsequent trials and ecological risk assessment, ethics, public perceptions, and regulatory requirements (Lavery et al., 2008; WHO/TDR, 2014; Brown et al., 2014). Researchers can draw from the advice of individual experts, advisory panels, personal experience, funding agency policies, and published findings to establish decision points. It is unlikely that one site will meet all of the criteria that are initially considered, and so a set of core criteria may need to be agreed upon to help with selection. WHO's Guidance Framework for Testing of Genetically Modified Mosquitoes (WHO/TDR, 2014) suggests that the criteria for contained field trials should include spatial location (for example, an island to mitigate the movement of organisms outside of a study area). Lavery et al., (2008) identified the ability to gain access to communities and their administrative authority as a criterion. Brown et al., (2014) argued that such criteria should include the expertise of a research team in-country, a credible regulatory structure appropriate for research activities, and the presence of target wild-type species, among others. The set of reasonable potential locations may expand or shrink as more information is gathered. Where research infrastructure is lacking, for example, opportunities for capacity-building as a direct result of research funding could be considered, such as occurred with the TARGET MALARIA Project.[2]

Phase 1: Laboratory-Based Research

Phase 1 research on gene-drive modified organisms will be performed in the laboratory and physically contained settings under highly controlled conditions. Testing during this phase will inform researchers on the efficacy and safety of the technology in laboratory populations, including whether the gene-drive modified organism demonstrates the molecular, biological, and functional characteristics desired for the chosen application. Physically contained trials will also allow the collection of necessary behavioral data to inform future research phases. Phase 1 research will encompass incremental studies from understanding the biology of the gene-drive modified organism to testing under contained conditions. An

[2]http://targetmalaria.org; Adelman, 2015.

example of a "go/no-go" decision tree to help researchers transition from one part of the research to the next is provided in Figure 5-2.

Keeping in mind future efficacy and safety, one important focus of Phase 1 is the optimization of containment settings for gene drive research. In addition to standard bench research, studies can be performed in physical containment that includes small cages, greenhouses, growth chambers, or aquaculture tanks. The choice of containment strategies will depend in part on the species in which the gene drive will be developed and on regulatory or other requirements from the research institution. Similar to good laboratory practices that include procedures to control for unintentional harm of technical staff in the laboratory, training of personnel on standard protocols for using and maintaining small-scale cages and other such facilities are required to prevent unintentional releases.

Another essential component of phase 1 research, *i.e*. before the organism is release into the environment, is to study bioconfinement and molecular-based strategies to mitigate harm caused by unintended release of organisms. These will be essential tools before research progresses to phase 2. Examples include the development of a reversal drive—the currently theoretical process by which the effects of a gene drive are reversed, using either the process that triggered the original gene drive or another process as yet undeveloped—to remove, replace, or restrict activity of the gene drive constructs in the modified organisms would have to be considered in phase 1.

It is also crucial to develop appropriate methods to minimize off-target effects that may reduce the ability for the gene drive to spread into the population is also essential., For instance, off-target effects could be controlled by carefully designing specific guide RNAs, optimizing endonucleases, and maximizing DNA repair mechanisms to increase the precision of editing (see further details below).

Also, phase 1 includes laboratory experiments designed to evaluate the stability of the gene drive construct (that is, whether the gene drive construct behaves in a predictable way across generations), and the fitness of the organism (its ability to survive and reproduce) in order to set the baseline population-level effects, and non-target effects. Where possible, organisms studied in phase 1 will possess a similar genetic background as the targeted wild-type organisms to help inform evaluations of the gene drive organism's fitness and behavior, and to provide datasets that may be required as part of the TPP. Such datasets can also help anticipate interactions within the open environment, such as predator-prey relationships (Hurst et al., 2012).

TABLE 5-1 Hypothetical Target Product Profile (TPP) for a Gene-Drive Modified Organism

Specification	Minimum Threshold
Gene drive construct uptake	>95% uptake in target species
Off-target effects	
• Organism survivorship	>98% in target species
• Mating competitiveness	at least 5% greater than unmodified male
Hybridizatiwith sympatric species	<1% over 10 generations
Interaction with existing applications	No change in efficacy of existing application
Impact	>60% reduction of target population
Time to impact	No greater than 1 year after release
Throughput	Two releases per day in target area by one technician
Deliverability	Delivered using existing health system
Training	Can be deployed by community volunteer
Cost at full scale deployment	No greater than current standard technology
Manufacturing	Meets demand

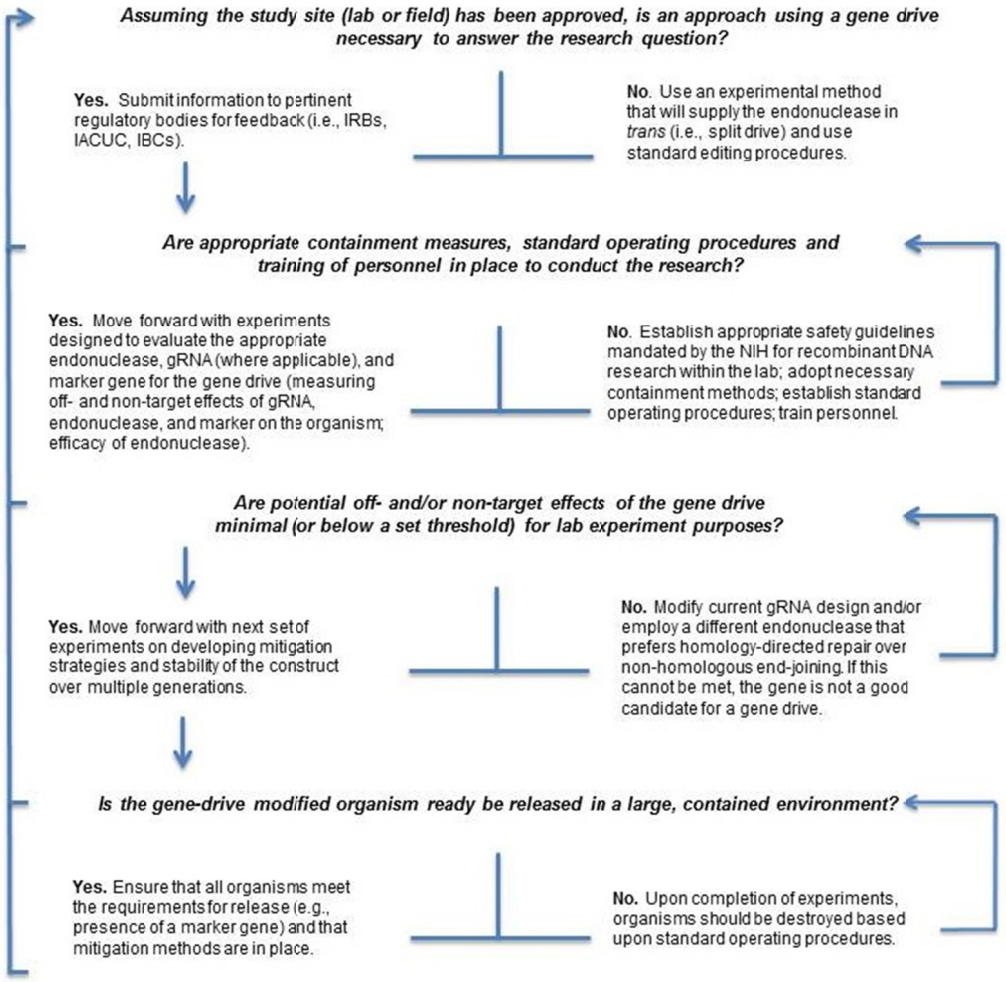

FIGURE 5-2 Hypothetical decision tree to guide the initial phases of research on gene drives. Adapted from the "Elements of the critical path for GMM development and deployment" section of the WHO for Guidance Framework for Testing of Genetically Modified Mosquitoes (WHO/TDR, 2014).

Phase 2: Field-Based Research

Phase 2 (Field-Based Research) involves studies in natural settings under conditions where dispersal or persistence of the organisms outside the evaluation area is restricted. Field-based research can take place in areas with natural barriers, such as islands which constitute an ideal geographically isolated contained setting, where climatic and environmental conditions are similar from where the organism would normally thrive while physically limiting the dispersal of the organisms (O'Connor et al., 2012). Other examples of research that could be considered in phase 2 are small-scale ecologically or biologically confined field testing of gene-drive modified organisms. Confined field testing entails methods than can control the persistence of an organism in the environment. This can be done by spatial isolation, such as a controlled release occurring at a set distance away from households or in a specific environmental niche (Suwannachote et al., 2009). This can also include climatic isolation where the surrounding environment would be suboptimal for organism survival given a set threshold if unintentional release occurred (Akbari et al., 2015, Adelman, 2015) or even through the use of chemicals to alter specific biological functions in gene-drive modified organisms to reduce their viability (Phuc et al., 2007). Gene-drive modified organisms could also be "field" tested in outdoor large-scale but physically contained environment such as large screen-house facilities (Benedict et al., 2008, Ferguson et al., 2008, Facchinelli et al., 2011).

As for phase 1, phase 2 research are also intended to validate the assessment of the biological and functional activity of the gene-drive modified organisms, but under more natural conditions, and will include the measurement of consistent behaviors, population-level effects and effects of the gene drive on wild-type organisms from the same species or non-target species of specific interest (i.e., beneficial organisms, organisms that may be closely related). The considerations about what endpoints to measure are made among stakeholders prior to seeking regulatory approvals.

As develop in phase 1, physical marking of test organisms (described later in this chapter) needs to be conducted to help study staff recognize gene-drive modified organisms (Handler et al., 2001). Evaluations in large outdoor cages or screen-houses could include post-test capture of test organisms using manual collection devices or traps to control for unintended release to the outdoor environment. If trials include open field releases into geographically contained or ecologically confined environments, investigators can inform community members immediately surrounding the test area so that they can report organism sightings; meanwhile, the study staff can employ appropriate methods for monitoring and collecting any escapees. Examples of measures that can be employed to control unintentional release or escape beyond the test area, or if required to 'stop' the trial for safety or regulatory reasons, might include manual collection techniques (such as aspirators or trapping devices), fumigation with insecticide, or treatment with rodenticides. However, it will be important for investigators to have characterized the resistance profile of test organisms if chemicals are considered as a mitigation strategy (Endersby and Hoffmann, 2013).

The decision about requirements for phase 2 testing conditions for a gene drive will be based on discussions during phase 0 regarding safety and efficacy and will be made in conjunction with the appropriate regulatory authorities (such as authorities with jurisdiction over public health, agriculture, and other areas) and local communities where the field testing will occur. Requirements for obtaining testing approvals will depend on many factors including the application of the gene drive technology and prior knowledge of the potential effects on the receiving ecosystem, and other factors that will be taken into account in risk assessment (see Chapter 6). However, the regulatory requirements for field-based research are expected to be different depending on the application of the research and the study site, since an ecologically confined field trial for gene-drive modified organisms involves intentional, although limited, release into the environment.

Phase 3: Staged Environmental Release

Phase 3 will involve a series of releases into an open environment. Initiating these larger trials and open-environment releases will require thoughtful, evidence-based decisions by a range of stakeholders applying criteria thresholds of the TPP as well as the application of relevant ethical and regulatory practices (see Table 5-1). Phase 3 trials will also include evaluating the release of the technology to inform capabilities and capacity requirements for full implementation and surveillance of the gene-drive modified organisms in phase 4.

As with phase 2, research on phase 3 may also focus on the fitness of gene-drive modified organisms under natural conditions, including elements such as climate fluctuations or variations in target-species densities that may affect the overall performance of the organism. As opposed to non-driving technologies, which can be limited by parameters such as population size or study duration, gene-drive modified organisms will likely persist in nature. Phase 3 studies will therefore help refine parameter thresholds, that once reached, that will allow the gene drive to spread throughout the wild-type population. To that end, characterization of the population structure of wild-type organisms of the same species as gene-drive modified organisms in the setting where testing will occur will be important to guide study design related to release rates (e.g., density and timing), as well as expectations of gene drive spread based on estimates of population size in the open field environment (Jeffery et al., 2009).

While measurement of effects as pre-defined in the TPP will likely remain the focus of the staged environmental release, the measurement of the impact of the gene drive on other populations within the ecosystem is also important component of phase 3. For example, trials requiring the demonstration of an

epidemiological impact (e.g., reduced disease prevalence, population suppression, or recovery of a threatened species population) can be used to inform decisions about whether the gene-drive modified organism could be released in other countries.

Phase 4: Post-Release Surveillance

The final phase of the testing pathway encompasses surveillance and monitoring. The purpose of this phase is twofold: (1) to determine whether intended effects of the broad scale release of gene-drive modified organisms are sustained over time; and (2) to detect any changes in the organisms or the ecosystem. For example, in a release program of gene-drive modified mosquitoes unable to carry the avian malaria parasite (Case Study 3) it would be important to monitor for the presence of the mosquitoes and confirm that they continue to be unable to carry the parasite. Also, long-term surveillance of honeycreeper population size and health will be needed. As noted in WHO (2014), efficacy can change due to changes in genotype of the organisms, or due to external factors such as weather or human activities. In addition, there could be unexpected effects when the gene-drive modified organisms are released (or expand) into new areas.

Monitoring also helps to determine whether any changes are needed in management of the gene drive (e.g., the possibility that mutations in the gRNA could arise over generations, leading to other recognition sites that were not detectable in early-phase testing), the gene-drive modified organism, and the release program (e.g., coverage, frequency, and density), or other aspects of an integrated program (e.g., the use of a complimentary, alternative strategy). It will also be important to continue to assess public support through surveys and other social science research tools (Hanh et al., 2009).

Longitudinal monitoring over varying time periods may be required to build robust and informative datasets regarding the effect of seasonal changes on the biology, behavior, and species composition of wild-type organisms within the target ecosystem (see Chapter 2). Simulation modeling of existing datasets, and those generated during research, will be an important component of research using gene drive technology (Marshall and Hay, 2012; Dutra et al., 2015). Open-access data repositories and standard operating procedures will facilitate the use of such data and models and inform standards for research design and monitoring schemes for gene drive research. In one example, Crain et al., (2013) used existing data from field research and "a modeling analysis to predict the dynamics when two *Wolbachia* infection types do not remain geographically isolated".

Monitoring and surveillance are necessary to determine whether the approach continues to work over time, but these activities can be expensive and logistically challenging, particularly for low- and middle-income countries. Thus, it will be important to select the measurement tools, timeframes, and protocols that are most informative and sustainable.

CONTAINMENT, CONFINEMENT AND MITIGATION STRATEGIES

Selecting or developing appropriate confinement and containment strategies is challenging due to the wide range of proposed gene-drive modified organisms. The case studies discussed in this report focus on mosquitoes, mice, and two species of plants. Certain mitigation measures may be an option for some types of research or certain organisms but not to others. For example, creating a gene-drive modified mosquito susceptible to insecticide might be a useful mitigation precaution, for which there would be no parallel with another type of gene-drive modified organism. Unless otherwise specified, the following sections focus on strategies that could potentially be used for any type of organism.

Two important dimensions of research carried out through the phased testing pathway are:

1) Containment and confinement to reduce the potential for unintended release or persistence of gene-drive modified organisms, respectively; and
2) Mitigation strategies to address potential harmful off-target and non-target effects.

Given the recent recognition by many scientists that CRISPR/Cas9 technology likely holds the greatest promise for rapidly creating gene drives in the laboratory for deployment in the field (Oye et al., 2014; Akbari et al., 2015), the considerations outlined in this pathway are primarily geared toward this technology. However, some of the same principles can be applied when using other gene drive methods described in Chapter 2.

Methods and strategies considerations for the choice of confinement and containment measures will include whether the organism will be evaluated only in the laboratory (phase 1) or in an open environment (phases 2-4).

It is important to highlight that while some effects could be harmful, some off-target and non-target effects could also potentially be beneficial, and some effects, such as cost to fitness, can be viewed as beneficial or harmful depending on the objective of the gene drive strategy. For example, regarding population suppression, a slight reduction in fitness could be considered unimportant or perhaps as a modest benefit (for example, a gene drive to reduce the population of a pest species). However, a reduction in fitness in the context of population replacement could be considered detrimental (for example, a gene drive intended to prevent a species from going extinct). Another important reason to mitigate off-target effects is that they may confound results obtained with gene drives, making it difficult to attribute phenotypes to the edited target. However, not all off-target effects are considered equal, and the number of off-target editing events may not be as important as the identity of these events (Mathews et al., 2015; Church and others personal communication, Human Gene Editing Summit, Dec. 1-3, 2015[3]). It is crucial to consider the functional consequences of off-target effects and whether their presence is detrimental or advantageous with respect to the purpose of the gene drive.

In addition to considering off-target and non-target effects, it is important to characterize the biology and ecology of the target organism and its environment to fully understand and control for these unintended effects (see Chapter 2). It will be important, for example, to support characterization studies over multiple generations to inform models of organisms' behaviors and properties before moving to field-based studies. Such research is critical for developing effective gene drive applications in various ecological contexts and for reducing uncertainty via informed risk assessment (see Chapter 6).

This section below outlines various confinement, containment, and mitigation strategies for consideration in gene drive research, as well as mitigating other types of concerns such as "tinkering with nature" or "who gets to decide whether a gene-drive modified organism should be released" concepts highlighted in Chapter 4 (Values), Chapter 7 (Engagement), and Chapter 8 (Governance).

Containing and Confining Gene-Drive Modified Organisms

In general, confinement and containment requirements will be worked out on case-by-case basis in consultation with an Institutional Biosafety Committee (IBC) or equivalent institutional research oversight body. Carefully discussing containment and confinement measures during phase 0 is crucial since organisms containing a gene drive will, by essence, spread the gene drives if released in an environment that promotes their survival and reproduction. In order to prevent lab-based gene drives from escaping into wild populations, many researchers have offered suggestions for developing methods to contain gene-drive modified organisms (Esvelt et al., 2014; Oye et al., 2014). The following containment methods could be used for gene drive studies in the laboratory. These containment mechanisms are also applicable to gene drives designed for various stages of field release (see phases 2-4) and are an important component of any mitigation strategy.

A split gene drive may be equally as effective as intact gene drive methods for modifying an organism's genome through germ line transmission (such that all cells are edited), while increasing containment capabilities. In a split gene drive, the components (Cas9 or other HEG, gRNA, and donor template; see Chapter 2, Figure 2-2) are supplied separately to the organism. With this technology, a gene drive is not actually created due to the manner in which the components for the editing are delivered to the organ-

[3]http://www.nationalacademies.org/gene-editing/Gene-Edit-Summit/index.htm.

ism. This method is particularly useful when performing standard editing techniques to alter specific genes as would have been carried out previously using more "traditional" methods. For example, one could use organisms transgenic for Cas9 (or the gRNA) and supply the other component independently, along with any donor DNA that might be required to modify the organism. This type of experiment has been successfully carried out in *Drosophila* (CRISPR-it; Port et al., 2015) and yeast (DiCarlo et al., 2015) and may be applicable to other organisms, especially plants and animal models of disease where transgenics are possible or in which gene editing is feasible. Although there is a small possibility that these individual components could recombine and create a gene drive, this possibility is remote and would not preclude the use of this system in the laboratory. Nonetheless, it will be important that the general considerations for gene drive usage in the laboratory, as outlined above (see Figure 5-2), be followed, particularly with respect to the choice of endonucleases, gRNAs, and measurement of off- and non-target effects, and employment of specific containment methods, standard operating procedures (SOPs), and training protocols. If the creation of an intact gene drive is required, perhaps due to limitations associated with the use of a split gene drive in a particular model system or because the ultimate goal builds toward environmental release (as in the case studies), then guidelines listed in Table 5-1 and described in detail below will also be important for researchers.

For organisms with a gene drive used exclusively in the laboratory and not intended for release, containment strategies may be minimal if appropriate mitigation strategies are employed (see next section). To this end, researchers are encouraged to follow principles of Good Laboratory Practices (GLP), including, for example, an internal monitoring system based on IBC feedback, as well as training for staff, researchers, and students in necessary SOPs. The training might also include specific instruction about the ecological differences between transgenic and gene-drive modified organisms.

If a specific marker can be visualized (e.g., using fluorescent proteins; see below), all personnel will need to see examples of the modified organisms to avoid confusion with other organisms without the gene drive and provided with appropriate materials, such as vials or cages, for collecting test organisms found outside of their normal area. Physical marking of adults, such as the use of fluorescent proteins, can allow for easy visualization of the research organism being studied (Hagler and Jackson, 2001). Reporting notices for the sighting of these organisms can be posted on office or laboratory doors. Keeping a form with contact numbers and sighting dates in work spaces will facilitate the ability of laboratory staff to report identification of any collected specimens to IBCs (as specified), and follow-up with resolutions to containment breaches, which includes informing surrounding laboratories of accidental releases. If live organisms are used in phase 1 testing (e.g., to identify variation in mating or other behaviors), traps are recommended in testing laboratories and rearing facilities to facilitate capture of specimens that have escaped or were released unintentionally. The U.S. Department of Agriculture has developed guidelines for containment that are expected to apply to gene-drive modified organisms and research under laboratory testing conditions (APHIS, 2002)[4]

Containment and confinement measures can be categorized as being extrinsic (e.g., in the laboratory or in the ecological or geospatial environment) or intrinsic (e.g., molecular or reproductive factors) with respect to the gene-drive modified organism (Esvelt et al., 2014; Akbari et al., 2015). Gene drive research regulations will most likely fall under the Coordinated Framework for the Regulation of Biotechnology as it also regulates genetic engineering in general. As such, gene drive research would receive oversight from IBCs. This is reviewed in detail in Chapter 8 on Governance.

Extrinsic physical containment of organisms in the laboratory, as outlined in the current National Institutes of Health guidelines for organisms containing infectious agents, in the Coordinated Framework for the Regulation of Biotechnology or in the Arthropod Containment Guidelines[5] can follow standard Arthropod Containment Level 2 criteria in the case of mosquitoes or other more stringent criteria depending upon the type of ecological confinement used, the organisms involved and the purpose of the experiment. These guidelines may be sufficient to conduct research with organisms containing gene drive con-

[4]https://www.aphis.usda.gov/plant_health/permits/downloads/arthropod_biocontrol_containment_guidelines.pdf
[5]http://www.ehs.wisc.edu/bio/ArthropodContainment.pdf.

structs in the laboratory. Methods of physical containment may include conducting experiments in a biosafety cabinet or in a separate room with a double-door entryway; the use of appropriate directional air flow; the use of air cloths or curtains (where appropriate); storage of tubes of gene-drive modified organisms on a separate bench, refrigerator, or freezer; housing of gene-drive modified organisms in cages or tanks separate from their wild-type counterparts (and in different rooms); installation of rodent-proof doors; securing plates of gene-drive modified organisms with parafilm; and, upon completion of experiments, destruction of all materials through autoclaving, freezing or microwaving (Akbari et al., 2015). Other standard laboratory practices would also apply here, including wearing personal protective equipment such as lab coats and gloves and appropriate clothing; cleansing benches with 70% ethanol upon completion of experiment; and soaking of glassware for 24 hours in Wescodyne solution before cleaning (Akbari et al., 2015).

Ecological confinement methods are also recommended to help prevent gene-drive modified organisms from mating with organisms in the native population or persisting in the context of the environmental conditions or geographical location of the laboratory. For example, this might involve working with species that do not normally survive in the region where research is being conducted; however, this might not be feasible in all instances as it could prevent research on gene drives from being conducted.

Intrinsic confinement and containment measures are also important. For example, the gene-drive modified organism could exhibit a barrier to reproduction such that it cannot mate with organisms in the wild. Additional methods of molecular containment can be explored, including the use of a split gene drive in which Cas9 is introduced separately (e.g., on a plasmid) from the gRNA (see above). Providing Cas9 (or other endonucleases) in *trans* has been successful in generating gene drives in yeast (DiCarlo et al., 2015) and *Drosophila* (Port et al., 2015), but this is likely to be species- and locus-dependent. One advantage of this method, is that less strict extrinsic confinement and containment measures would be necessary, as these organisms are considered standard transgenic animals and are thus subject to regulations already in place. Another approach is to design gene drives to be "self-limiting", for example, by carrying both a gene that encodes for a toxin and another gene that confers immunity to the toxin. Such gene drives could self-destruct either over time or upon addition of a chemical (Gould et al., 2008, Marshal et al., 2012). One final intrinsic containment mechanism is to target sites for which the gene drive is only found in a laboratory organism and not in wild-type organisms.

Mitigating Potential Harms

Restoration of wild-type organisms

When the intent of the gene drive is population replacement, restoration of the wild-type version of the sequence edited by the gene drive (including off-target effects) may be desired or required. One mitigation method that addresses this issue and has been demonstrated exclusively in the laboratory is the use of a reversal gene drive (DiCarlo et al., 2015). This method is based on the use of another gene drive that re-introduces the original genetic sequence into the edited organism, along with modifications to it such that it can no longer be edited in the future. This method requires a two-step modification of the organism through the use of two rounds of editing (i.e., introduction of two different CRISPR/Cas9 systems). Another mechanism proposed is an immunization drive that, when given to an organism, will prevent a second gene drive from being propagated within the organism by altering sequences targeted by the second drive. The immunization drive could be deployed so that non-target species do not inadvertently receive the gene drive. It is important to note that with either of these methods, Cas9 and the gRNA would still remain in the genome, which could cause additional undesirable effects due to persistent DNA breaks caused by Cas9. Another strategy is to adapt a new transgene system developed in *Drosophila* called Cas9-ablated chain termination, where possible (Wu et al., 2016), which serves as a molecular "brake" to cleave Cas9 and thus disable it in Cas9-containing organisms, thereby rendering the gene drive inactive. Finally, one could maintain a population of wild-type organisms that, upon disabling of the gene-drive

modified population through any of the methods described above, could be released to re-establish the native population.

Redressing undesirable ecological and evolutionary consequences

For redressing undesirable ecological and evolutionary consequences, a strategy could include monitoring specific non-target species alongside the gene-drive modified organism. For timely recognition of undesirable ecological consequences, the best approach is to monitor the densities of species most closely linked to the target species via trophic connections (e.g., competitors whose diets overlap that of the target species or predators that might prey on the target species). One of the most likely undesirable evolutionary consequences would be the movement of the gene drive into a closely related, non-target species via reproduction between two different but related species (i.e. hybridization). Close evolutionary relatives could be monitored in the wild for the appearance of the drive unless hybridization is known to be impossible (i.e., if resulting hybrids do not produce fertile eggs). A potential example is Palmer amaranth (see Case Study 6), which has been shown to hybridize with other species. The interventions for both types of consequences could include the re-introduction of affected species after the gene drive has been eliminated. In both cases, the speed with which gene drives can spread suggests that monitoring must be in place before the gene drive is introduced so that any unwanted effects can be recognized quickly (see description of phase 3 and phase 4 activities above). This is especially important in the context of potential ecological consequences of a suppression gene drive, because the loss of a species can, in some cases, produce effects that cascade through the ecosystem (Estes et al. 2011). These kinds of effects can be reversed (e.g., Shapiro and Wright, 1984) even by re-introduction of the lost species (Bundy and Fanning, 2005; Mumby and Steneck, 2008). Depending upon the reproductive capacity of the edited organism (e.g., its generation time), it may take some time for all organisms within the population to have the original phenotype restored. Maintenance of a copy of Cas9 and a gRNA could also have deleterious effects over time on the organism and other non-targets. Non-target effects may also be hard to control, and redressing potential undesirable ecological and evolutionary consequences of the gene drive, even when accounting for changes over time, may be difficult. These issues are discussed in detail in Chapter 2.

Optimization of gRNA design

Off-target effects are going to occur with any gene editing methods associated with homing endonucleases that involve the creation of breaks in the DNA (e.g., Cas9, ZFNs, TALENs) as well as gRNA hybridization (CRISPR/Cas9). However the rate will likely be organism or cell type-dependent. In order to mitigate such effects for RNA-guided editing, it is critical to optimize gRNA design. To achieve high specificity, evaluation of the target DNA to identify sites for gRNA hybridization is an important step. If the target lacks specificity (i.e., if the DNA sequence resemble others in the genome) then other sequences in the genome will be targeted. Likewise, chromosomal rearrangements after imprecise repair will occur, which may trigger the activation of aberrant signaling leading to cell dysfunction (Koo et al., 2015).

To mitigate harms related to off-target effects of gRNAs, scientists have used web-based bioinformatics tools. These tools help assess the degree to which the gRNA(s) may target other sequences within the reference genome of the chosen organism and the genomes of other organisms. This is only possible to do, however, if genomic sequences of targeted and non-targeted organisms are available. Targets that have few or no closely related sequences in the genome can also be chosen.

If the gRNAs are specific (i.e., if the intended phenotype does not change over time), and if any change in fitness does not prevent the spread of the organism, then the gene drive has a chance to be successful in the wild. Re-introduction of the wild-type or original allele can also be undertaken to ensure that the phenotype in the presence of the gene drive is attributable to the editing of that allele (Bono et al., 2015). A powerful way to complement computational methods is the use of Next Generation Sequencing (NGS), which can generate a genome-wide profile of the nuclease activity. Once the putative off-target sites (i.e., sites that resemble the targeted DNA sequence) have been detected computationally, these sites

are compared to the presence of nuclease activity identified at these specific sites by NGS. However this technique introduces some "observational bias" based on the assumption that off-target sites will resemble the target site, while others can exist. Other considerations include the fact that sequencing-based assays can lead to artifacts (Koo et al., 2015) that may preclude actual detection of off-target effects (Mathews et al., 2015), and the fact that the configuration of the DNA may also impact whether potential off-target sites are even accessible to the nuclease (Sander and Joung, 2014; Koo et al., 2015). To address such constraints, a new NGS method called Genome-wide Unbiased Identification of DSBs Enabled by sequencing has been developed to physically tag all potential cutting sites, including off-target sites, in an unbiased fashion (Tsai et al., 2015). When compared to computational methods, the results using this sequencing method revealed that off-target effects were observed at higher frequencies than expected. Several groups have now used such tools and others to reveal off-target effects in various cell lines (Wang et al., 2015, Frock et al., 2015) including pluripotent human cell lines (Chan et al., 2015). Therefore, computational models to predict off-target sites and the use of NGS to profile the activities of human and animal model gRNA, are necessary to maximize activity of the gRNA while minimizing potential harmful effects (Doench et al., 2016).

Optimization of endonuclease cutting efficiency

Similar to the considerations for optimizing gRNAs described above, different endonucleases (e.g., Cas9 or other homing endonucleases) can vary in their ability to efficiently cut the targeted sequence. To this end, researchers have used a mutant version of Cas9, called Cas9 nickases, along with two gRNAs targeting two different sites, one on each side of the DNA strand. This endonuclease only makes breaks on one strand of the DNA as opposed to both strands (Ran et al., 2013); it also engages a higher-fidelity type of repair than the one used after a Cas9/gRNA-mediated cut is made. Other genetically engineered Cas9 variants (Nishimasu et al., 2014; Anders et al., 2014) cleave at different PAM sequences and/or with higher efficiencies and reduced off-target effects (Kleinstiver et al., 2015; Slaymaker et al., 2015). A new Cas9-like protein has now been identified, called Cpf1 (CRISPR from *Prevotella* and *Francisella* 1), that functions through the use of a single gRNA molecule; this protein generates DNA breaks in the form of overhangs (a staggered cut) instead of blunt ends, cuts at a greater distance from the PAM on the target site, and therefore does not disrupt the PAM upon cutting (Zetsche et al., 2015). These other Cas9 endonucleases have yet to be evaluated for efficacy and efficiency in living organisms. Funding for these latter experiments to address the efficacy and specificity of gene drives is critical for the future deployment of gene drives in plants and animals. Importantly, the presence of Cas9 carried in the organism will need to be evaluated to determine whether Cas9 has a harmful effect on organism fitness that would prevent the spread of the gene drive (discussed in Chapter 2), as this would raise significant questions regarding the ability of the gene-drive modified organism to function.

Optimization of homology directed repair (HDR) versus non-homologous end joining (NHEJ)

When DNA cleavage occurs at the targeted site, there are two major categories of DNA repair that can restore the DNA structure: homology directed repair (HDR), which requires a homologous sequence to guide repair, and non-homologous end joining (NHEJ), which does not need a homologous template for repair and just "seals" the cut DNA ends together. Depending on the application, gene drives may require the introduction of specific genes into the target chromosome and thus would require HDR. This could be one of the biggest challenges facing gene drives, because the mechanism of repair will depend on species, cell cycle stage, cell type, and stage of development (Esvelt et al., 2014).

In order to facilitate the HDR pathway and allow the introduction of an exogenous gene, Cas9 nickases (see above) can be used, since it cuts a single strand of DNA instead of the two strands. Similarly, the nuclease Cpf1 should (theoretically) more easily allow for insertion of DNA due to the presence of compatible overhangs. Other options involve the repression of genes involved in NHEJ or the activation of genes responsible for HDR (reviewed in Esvelt et al., 2014). For instance, to optimize HDR during the

development of their gene drive-modified mosquitoes, Gantz et al. (2015) and Basu et al., (2015) included dsRNAs directed to both Cas9 (on the construct) and a gene expressing a protein essential for the NHEJ activity in the mosquito. Upon injection into the mosquito, this gene drive construct silenced the Cas9 protein and reduced the activity of NHEJ in favor of HDR, allowing for the insertion of the entire gene drive construct in the genome. Recently, Hammond et al., (2016) observed a bias toward the HDR repair mechanism using the CRISPR/Cas9 technology in mosquitoes without such optimization but more research would be needed to confirm such results.

Evaluating the stability of the gene drive construct over multiple generations

Another challenge related to repair mechanisms is that gene drive resistant alleles may result when NHEJ repairs the break caused by homing endonucleases, leading to the loss of the cleavage site. Such alleles without the cleavage site will become resistant to the effects of the gene drive. If enough individuals contain the resistant allele, then the gene drive may become ineffective. One way to reduce the incidence of resistance would be to use multiple gRNA because resistance would require mutations at several target sites. A similar challenge stems from the fact that different DNA sequences for the same genes are found in nature (known as polymorphic sequences). This could prevent the action of a gene drive because the gRNA may not be designed to recognize such sequences outside the laboratory. If these "natural" resistance alleles are common in the wild, the gene drive may be ineffective.

The stability (or lack thereof) of a gene drive, indicated by the degree to which the modified genetic element and the driving capability are retained over multiple generations, needs to be determined on a case-by-case basis. To evaluate the gene drive's stability and to estimate its effectiveness, it will be important to carry out a variety of experimental assays, including the use of simulation modeling to predict the spread of the gene drive over multiple generations and any population-like effects using laboratory data (phase 1). These results can be compared to field data obtained from the non-driving study (see Quantitative Approaches, below) (Esvelt et al., 2014). For example, the gene drive stability will need to be measured in a stepwise manner first in the laboratory populations and then in wild caught populations. This is because there may be no perceived instability in the laboratory population, but potentially increased opportunities for instability in the wild population. If such instability arises in the wild, then there is no reason to take this gene drive outside of the laboratory to phase 2. The exception to this is when the gene-drive modified organism is being designed for field release for use in population suppression, such that any loss of organismal fitness could be advantageous for achieving the release objectives, as long as it does not affect the spread of the gene drive.

Determining the effects on organismal fitness

It is imperative to use quantitative methods to evaluate whether the expression of the homing endonuclease (for example, Cas9), the gRNA, or the cargo template (singly expressed or in various combinations) affect a gene-drive modified organism's fitness, relative to its wild-type counterparts. This evaluation would involve a "fitness assay" that would comparatively examine fitness parameters for the engineered genotype, relative to the unaltered wild-type organisms, ideally using established empirical methods in the particular biological system or a closely related one. In general, the fitness assay approach compares the relative ability for a test genotype to produce viable offspring to that observed with wild-type organisms, and the experiment is conducted with independent empirical replicates (Chippindale et al., 2001). This repetition is necessary to provide sufficient power for a statistical analysis to detect measurable fitness differences between the genotypes. In addition, it may be useful to gauge relative survival of the engineered genotype relative to the wild-type through replicated assays of relative lifespan (Rose et al., 1992), which statistically measure whether the genome alteration negatively impacts physiological health to shorten the average lifetime of the individual. These same types of assays will also need to be conducted for an organism in which the genetic alteration has been made using a different editing method, is found naturally in the population, or is created through genome-wide mutagenesis for all comparison

purposes. Although it is often assumed that genome alterations, including gene drives, will tend to negatively impact individual fitness relative to that observed in the unaltered wild-type organism (e.g., due to the addition of foreign DNA that slows replication, and/or interferes with native transcription and translation), this is an assumption that must be verified using rigorous empirical analyses. This hypothesis could be tested by performing fitness assays in the laboratory that measure the relative number and quality of viable gametes produced by gene-drive modified and wild-type organisms, as was applied by Hammond et al., (2016) to gene drive constructs in *Anopheles gambiae*, and by performing survival assays that compare relative viability of altered and gene-drive modified and wild-type organisms (Isaacs et al., 2012). Additional field trials can be used to examine these fitness effects under more natural conditions. The ability to quantify these effects on organism fitness, if not masked by compensatory pathways that are up-regulated by the organism as observed previously (Rossi et al., 2015), will lead to questions regarding whether gene drives provide the best technology for editing a specific gene, and whether fitness effects are consistent with intended applications.

Using visible markers

Gene-drive modified organisms that possess, as part of their genetic cargo, a marker gene in order to facilitate identification can help researchers distinguish a gene-drive modified organism from wild-type or other conventional transgenic organisms. Although still under development, examples include the addition of a gene encoding a fluorescent protein that would be expressed in a region of the organism that could be easily screened/monitored (e.g., eye, skin) without requiring sequencing assays which necessitate adequate equipment and expertise, are more invasive, and may take longer to obtain results. Alternatively, the gene drive could target an additional, non-essential gene for mutation to generate a visible phenotype that could be scored. These are both examples of common genetic marking techniques that have already been employed by researchers who have constructed gene drives in *Drosophila*, (yellow body phenotype in Gantz and Bier, 2015) and mosquitoes (white-eye phenotype and fluorescent marker in Gantz et al., 2015; fluorescent markers in Hammond et al., 2016).

The generation of unique labels for gene drive constructs, in the context of other conventional transgenic organisms possessing similar tags, could be problematic. The ability to do so will depend on the availability of specific promoter and enhancer combinations to drive marker expression in select cell types to allow for efficient and effective screening. For example, 95% of mosquito strains are labeled with only two fluorescent tags because the efficacy of expression of others is low, and there is currently a dearth of information regarding how other markers could be used in mosquitoes (M. Benedict, personal communication). This represents a significant challenge for the field. It is highly desirable to develop a consensus opinion within the community working on a particular organism with respect to how gene-drive modified organisms will be labeled and identified. Although not absolutely required, the inclusion of a visible marker is recommended when making a gene-drive modified organism.

Quantitative Approach to Evaluate Success and Impact

According to Sinkins and Gould (2006) "[m]athematical modelling can help to predict the utility of different gene drive systems, as long as realistic values for the fitness costs of the effector transgene and for the pest's population structure are used".

Quantitative and computational methods are vital tools for evaluating biological applications, and for advancing fundamental knowledge in biology. Often the overarching goal is to use bioinformatics, mathematical modeling and computer simulations to elucidate the dynamic properties of a biological system at one or more levels (e.g., gene, genome, population, community, and ecosystem). When this approach involves a probabilistic framework, it is possible to predict which factors are most likely to influence the success of biological applications and to reveal the variables that most influence dynamics in biological systems (Otto and Day, 2007). Such quantitative approaches can never incorporate all of the variables at play in biological systems because the mathematics quickly becomes too intractable or the

simulations exceed available computing power. Nevertheless, history shows that quantitative methods can usefully identify those variables that are most important in determining dynamic properties of biological systems, especially using an iterative process where empirical observations are employed to further refine the accuracy and predictive power of quantitative models (Kitano, 2002).

Gene drive technology is advancing quickly, and offers the possibility of an efficient tool to study fundamental questions in biology as well as a method to address problems in public health, conservation biology, agriculture, and other applications (Esvelt et al., 2014, DiCarlo et al., 2015, Gantz and Bier, 2015, Hammond et al., 2016). But the overall success and impact of gene drive technology hinges on many factors, especially when the strategy involves the release of genetically altered individuals into natural communities. The most proximate challenge is to gauge whether gene drive mechanisms such as gRNA editing are precise in altering only the target locus, versus inefficiently changing unintended (off-target) loci. If the goal of the gene drive technology is to alter genotypes for strictly laboratory-based basic research purposes, a certain level of inaccuracy may be tolerable. Still, if such experiments were intended to examine questions such as genetically inherited diseases in model organisms, any imprecision could confound assumed relationships between genotype and phenotype and thus slow the advance of knowledge. If gene drive technology inaccurately creates genotypes intended for field release, this outcome necessarily causes a disconnect between the expected introduction of individuals into the target population and the actual individuals that are placed in the wild. It may be impossible to absolutely know whether and how this proximate inaccuracy holds repercussions for overall success and environmental impact of the intended field release strategy, until the release actually occurs and the system is closely monitored. However, quantitative and computational methods should be useful in gauging the probabilities of success and possible outcomes, whether the drive is strictly laboratory-contained or intended for field release. For this reason, it is prudent for research on gene drive technology to include quantitative tools that help to refine the accuracy of their associated risk assessment frameworks.

As previously reviewed, current gene drive technologies mimic natural gene drive mechanisms (e.g., meiotic drive) that have been studied intensively, especially at the molecular and population biology levels (Jaenike, 2001). Similarly, biological control efforts are longstanding, and we possess knowledge of how released organisms can impact populations and communities (van Driesche and Bellows, 1996; Stiling and Cornelissen, 2006). Nevertheless, current gene drive technologies and their intended applications differ in several respects from naturally-occurring gene drive mechanisms and prior biological control efforts. For example, if a limited number of non-driving genetically modified organisms are released into the wild, this fundamentally differs from the release of gene-drive modified organisms because only the latter case involves sustained modification of individuals across multiple generations in the target population. Therefore, it would be naïve to assume that intensive quantitative modeling and other prior efforts would suffice to predict the accuracy of gene drive manipulations and determine how these altered genotypes would affect natural communities. This possible disconnect between prior knowledge and current goals of gene drive technology offers further support for the argument that quantitative and computational tools should be developed for each gene drive study because researchers should not assume that probabilities of success and environmental impacts could be drawn conveniently from prior data in a different biological system.

Quantitative approaches offer the opportunity to efficiently examine uncertainties related to the success and impact of gene drive technology, at all stages of research. Because monetary resources for basic research and for field trials can be very limited, quantitative tools also offer the opportunity to efficiently explore whether a genetic manipulation or field release may be successful, before actually devoting funds to conduct the work. In particular, this approach can be used to evaluate key steps in the phased testing pathways described earlier in the chapter, either at individual stages or more holistically across multiple stages. In this way, scientists can gain a broader predictive framework for whether the basic research goals can be properly advanced and whether the field release may truly work when launched.

Often these modeling approaches can assess key thresholds, such as how many individuals must be released for the gene drive strategy to likely succeed in sufficiently altering the target population. Similarly, a wide range of effects may be vital for predicting the success and impact of the gene drive technolo-

gy. Considerations may include: the predicted average fitness of altered individuals relative to the genotypes in the targeted wild-type population; how quickly or slowly should the altered individuals be released to maximize (or minimize) their impact in the environment; how current sex ratio and size of the target population may influence outcomes of release; and whether geographic barriers or other effects of landscape ecology will impact the likelihood of the gene drive spreading successfully.

LEARNING FROM FIELD RESEARCH AND BIOCONTROL EFFORTS WITH OTHER TYPES OF MODIFIED ORGANISMS

Due to the expectation that organisms will disperse in the open environment during phases 3 and 4, causing the gene drive to spread and potentially impact broader human and environmental communities, mitigation in these phases offers additional challenges to those described for laboratory (phase 1) and contained releases (phase 2). Past experience with biocontrol efforts and research with modified mosquitoes, such as Release of Insects with Dominant Lethality (RIDL®) technology and infection with *Wolbachia* bacteria, can inform questions about population biology and ecosystem dynamics when considering mitigation strategies for research using gene drive technology.

Biocontrol Pest Species

Biological control, defined by Popovici et al. (2010) as "the release into the environment of a biological agent to control a given pest through mechanisms such as predation, parasitism, herbivory or disease" of agricultural, livestock and human pests has been undertaken successfully for centuries (Wackers & van Rijn, 2007). Examples of the range of biocontrol applications from Australia alone were reviewed by Popovici et al., 2010 and "include the release of myxoma virus to control rabbit populations (Fenner, 1983; Saunders et al., 2010), the release of *Cactoblastis* moths to control prickly pear (*Opuntia* spp) (Dodd, 1940), the introduction of dung beetles to manage cattle dung and the bush flies that breed in it (Edwards & Pavri, 2007) and the control of floating *Salvinia* weed (Room et al., 1981) using the beetle *Cyrtobagous singularis"*.

Intentional Release: Large-Scale Deployment

Sterile insect technique (SIT), "a method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species"[6], continues to be employed on a large-scale to control the new world screwworm, *Cochliomyia hominivorax* (Knipling et al., 1955, Vreysen et al., 2007). SIT has also been used to control the Mediterranean fruitfly (also called the medfly) *Ceratitis capitata* and as part of an integrated pest management program. In addition SIT has also been employed to control the pink bollworm (*Pectinophora gossypiella*) since 1999, and during the cotton season, approximately 25 million sterile moths, reared at a facility in Phoenix, Arizona are released per day.

Using the SIT approach as its foundation, the genetically engineered technique RIDL utilizes transgenic insects with a conditional, dominant, female-specific lethal gene that inhibits female offspring from developing into adults (Thomas et al., 2000). Recently, successful transformation of the diamondback moth using the piggyback transposable element prompted the development of RIDL as a control measure for diamondback moths by the biotechnology company Oxitec (Martins et al., 2012; Kelland, 2015). The RIDL approach to diamondback moth control has been evaluated in both the laboratory and contained environments; field test are underway[7] (Harvey-Samuel et al., 2014; Waltz, 2015). The development of RIDL approaches for the control of agricultural pests and invasive species, like the diamondback moth, represent another tool for integrated pest management programs. RIDL mosquitoes have also been re-

[6]FAO: http://www-naweb.iaea.org/nafa/ipc/sterile-insect-technique.html.
[7]http://www.oxitec.com/agriculture/our-products/diamond-back-moth/.

leased in several countries, including the Cayman Islands, Panama, Malaysia and Brazil[8], to suppress local mosquito populations for dengue control (see Case Study 2).

Another biocontrol approach is the use of *Wolbachia* infection. Mosquitoes infected with natural *Wolbachia* symbionts have been released in the United States,[9] Australia, Indonesia, Vietnam and Brazil[10]. The bacterial symbionts in the genus *Wolbachia* are widely distributed in insects (Werren, et al., 1995, Werren and O'Neil, 1997; Stouthamer et al., 1999; Bourtzis and Braig, 1999) and are transmitted vertically from mother to offspring through a phenomenon known as cytoplasmic incompatibility (Ghelelovitch, 1952). Because only *Wolbachia*-infected females can successfully reproduce with infected males, all the offspring of infected females will carry *Wolbachia,* which can then spread quickly resulting in a large proportion of the local mosquito population eventually becoming infected. The use of *Wolbachia* infections is advantageous because it reduces the lifespan of insect hosts (Sinkins et al., 1997; Dobson et al., 2002; Ahantarig et al., 2011; Bull and Turelli, 2013) and confers resistance to infection with dengue and chikungunya viruses in *Aedes aegypti* (McMeniman et al., 2009; Moreira et al., 2009a; Bian et al., 2010).

This technology includes options for sustained releases similar to RIDL for population suppression; it addition, it offers the opportunity for the release of self-sustaining variants that could lead to population modification, for example, by reducing the mosquitoes' capacity to transmit specific pathogens.

Although these technologies have encountered hurdles during their development, protocols, strategies, and guidelines were produced in anticipation of the ultimate release of suitably engineered mosquitoes (Beech et al., 2009a, 2009b; Mumford et al., 2009), that include sequential steps from concept to the safe and responsible release of engineered mosquitoes. These steps include development of cage (contained) trials, community engagement, and considerations of relevant ethical, social, and cultural issues. Remarkably, from what seemed like a position of insurmountable challenges, almost all of the problems have been resolved. The Gates Foundation in particular has strongly supported groups to develop recommendations and protocols related to transgenic mosquito releases (Singer et al., 2007; Lavery et al., 2008; El Zahib-Bekdash & Lavery, 2010; WHO, 2010).

The approval to deploy transgenic *Aedes aeg*ypti using RIDL technology in Brazil for dengue control demonstrates that assessment of benefits and harms based on data gathered on the biology, ecology and planned mitigation strategies can support a favorable decision (see Case Study 2). The concerns addressed are anticipated to be similar to those of gene drive technology (WHO/TDR, 2014). For example, considerations include exposure to humans, the ability of the organism to have modified competency for pathogen transmission, the possibility of gene flow to other species, the likelihood of an increase in the population of other species due to the reduction of the target organism, other environmental impacts, and an assessment of the functionality of a designed mitigation strategy to minimize unintentional harm — in this case, the requirement of tetracycline in an aquatic habitat to suppress lethal gene activation (Phuc et al., 2007).

Unintentional Release: Transboundary Movement, Hybridization and Horizontal Transfer

Given the fact that neither dengue nor mosquitoes respect political boundaries poses important logistical considerations for the use of *Wolbachia*-infected mosquito releases or any other form of biocontrol. Given the fact that *Wolbachia* can spread not only through mosquitoes but also through the fruit fly *Drosophila*, it is expected that once infected mosquitoes are released, *Wolbachia* would then become established and would perhaps slowly spread (i.e., an introduction in Vietnam would therefore eventually spread throughout "mainland" Asia). If *Wolbachia* infected organisms are detected in a neighboring country that did not approve this specific anti-dengue strategy, it could create a legal problem between the involved countries. Recognizing this issue, studies in Australia have monitored neighboring areas for the

[8]www.oxitec.com.

[9]www.scientificamerican.com/article/fighting-mosquitoes-with-mosquitoes.

[10]www.eliminatedengue.com.

potential spread of biological agents outside the study area. While, infected larvae were only detected beyond the study cite on just three occasions, the issue of permanent establishment could not be answered with any certainty with the current data and would need further investigation.

Likewise, a key consideration for gene drive development is the possibility of horizontal transfer (for example, the transfer of a gene drive construct to a predator or humans), which could lead to unpredictable non-target effects and unintentional spread of the gene drive construct in non-target organisms. Similar concerns were raised during the development of *Wolbachia*-based biocontrol techniques (Popovici et al., 2010); as a result, the example offers insights that could be useful for consideration of gene drives.

Early in the *Wolbachia* biocontrol research process and well before release, investigators engaged the community to identify major questions that needed to be addressed. The process resulted in discussions in three major areas:

- *Could Wolbachia affect/be transferred to humans via the insect saliva during blood-feeding?* In order to address this, phase 1 studies were performed to detect the presence of *Wolbachia* in the saliva of the *Aedes aegypti* mosquito (Moreira et al., 2009b). DNA amplification of *Wolbachia wsp* genes in the mosquitos salivary glands confirmed the presence of *Wolbachia* in the glands but the bacteria was absent in the mosquito saliva.
- *Could Wolbachia be transferred to another similar mosquito species?* Whether *Wolbachia* could be transferred to other organisms or become established in the soil was addressed using both experimental testing and indirect evidence. The former included the attempt to transfer *Wolbachia* in new species such as from flies into mosquitoes. The results indicated that the horizontal transfer of *Wolbachia* between these species was difficult and therefore considered negligible. The latter was based on the fact that since in Australia *Wolbachia* has been present in *Aedes Notoscriptus* it could have possibly been transferred to *Aedes Aegypti*. However, this transfer has never occurred.
- *Could Wolbachia be transferred into the environment?* A number of studies were conducted to evaluate if *Wolbachia* could be horizontal transferred into the surrounding environments where mosquitoes would be released. Predation experiments using spiders were performed in the laboratory (phase 1). To verify that *Wolbachia* did not disseminate in the environment a semi-field fully enclosed outdoor greenhouse designed and constructed specifically for the project was used (i.e., phase 2 [11]). Thousands of *Wolbachia*-infected mosquitoes were introduced with samples of plants, soil, earthworms and millipedes (to fully represent ecosystems in which *Wolbachia* could have propagated). These samples were then collected from inside the enclosure and tested by PCR for the presence of the specific IS5 *Wolbachia* genes but none were detected, indicating that no transfer of *Wolbachia* to other species had occurred. Additional studies of horizontal transfer by other investigators also supported this conclusion (Hurst et al., 2012).

CONCLUSIONS AND RECOMMENDATIONS

Although the potential for gene drives to address and solve problems associated with vector-borne diseases, invasive pests, and agriculture is truly exciting, before field testing or environmental release of gene-drive modified organisms, it is crucial to establish a rich understanding of the target organism, its relationship with its environment, and potential unintended consequences, such as off-target and non-target effects.

A phased testing pathway, such as the one developed by the World Health Organization for testing genetically modified mosquitoes, can facilitate a precautionary, step-by-step approach to research on gene drives. Each step in such a pathway promotes careful study and evaluation, includes a series of checkpoints to determine whether and when research should move to the next phase before proceeding to the

[11] www.mosquitoage.org/en/HOME.aspx.

next step, and provides vital data and knowledge that can be used to inform and enhance the effectiveness of other phases. A phased testing framework to guide step-by-step evaluations, of genetically modified mosquitoes, can be adapted for laboratory and field research on gene-drive modified organisms.

> Recommendation 5-1: Scientists conducting research on gene drives should follow a phased testing pathway, a step-by-step framework that begins with developing a research plan and continues through, if applicable, monitoring gene-drive modified organisms in the environment. Each phase in such a pathway should include pre-defined "go/no-go" decisions for determining whether to transition to the next phase based on evidence regarding harms and benefits, efficacy, and safety.

The goal of a gene drive is the rapid spread of genetic information throughout a population. This makes it especially important to minimize potential unintended consequences. Containing or mitigating unintended effects may require a combination of physical containment and biological confinement strategies. When developing biological confinement strategies, consideration will need to be given to their benefits, costs, and weaknesses or potential unintended consequences. For example, adding a visible marker to gene drive-modified organisms in some cases could have negative consequences for the organism, which will need to be weighed against the benefits of this strategy. It is particularly imperative to use caution when considering the development of a "reversal drive"—a gene drive designed to mitigate the unintended consequences of another gene drive—as it may be impossible to effectively employ this strategy without off-target effects or to fully redress ecological and environmental effects from the original gene drive.

> Recommendation 5-2: Whenever possible researchers should use available datasets and models to develop and evaluate strategies to minimize the potential for harmful off-target and non-target effects throughout the phased testing pathway.

> Recommendation 5-3: Whenever possible, researchers should use a split gene drive in laboratory studies to avoid issues associated with a failure of containment.

> Recommendation 5-4: Whenever possible, researchers should include a gene drive that spreads a visible marker to distinguish modified organisms and facilitate research and monitoring.

> Recommendation 5-5: Researchers, regulators, and other decision-makers should not rely upon a "reversal" gene drive as the sole strategy for mitigating the effects of another gene drive.

After release into the environment, a gene drive knows no political boundaries. It is desirable to expand the intellectual capital and research capacity of relevant institutions around the world to facilitate appropriate knowledge exchange and research collaborations pertaining to gene drives. In particular, this includes building long-term relationships with scientists in low- and middle-income countries where field research on gene-drive modified organisms is most likely to occur.

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6

Assessing Risks of Gene-Drive Modified Organisms

Advances in the molecular biology of gene drives are far outpacing research on the fate and effects of gene-drive modified organisms in the environment, as well as the development of the knowledge needed to calculate risk and describe uncertainty related to gene drives. There are many questions that need to be answered about the effects, both beneficial and harmful, that gene-drive modified organisms may have if released into the environment. For example, will the frequency of inheritance of the genetic construct remain constant from one generation to the next? What is the possibility for gene flow to non-target species? How reliable are molecular markers, such as adding a unique eye color, intended to facilitate the monitoring of gene-drive modified organisms after they have been released to the environment? What constitutes an adequate mitigation strategy for unintended, harmful effects, and how can the efficacy of such an approach be evaluated?

Although as of May 2016 many applications have been proposed, there has not yet been field tests or environmental releases of gene-drive modified organisms**.** Decisions will need to be made about prospective applications of gene drive research, including the direction of research, the need for public engagement, and the requirements for governance. Given the lag between this new technology's development and experts' understanding of its ecological implications, decision-makers' ability to identify the potential harms for different applications and determine appropriate safeguards and mitigation strategies is somewhat limited. How can decisions be made under such conditions of uncertainty?

The answer is *ecological risk assessment*, the study and use of probabilistic decision-making tools to evaluate the likely benefits and potential harms of a proposed activity on the wellbeing of humans and the environment, often under conditions of uncertainty. The scientific assessment of risk is one important way in which values related to protecting and preserving human health and the environment are incorporated into decision-making, particularly, when such assessments are mandated by law. This chapter focuses on why and how ecological risk assessment should be used to inform decisions around the development and application of gene-drive modified organisms, from understanding the efficacy and safety of gene drives created in the laboratory, to validating assessments in contained field trials, to assessing the risks of releasing gene-drive modified organisms into the open environment.

WHAT IS RISK?

The definition of risk varies depending on the context in which the term is used. In colloquial use, the term risk is synonymous with threat, harm, or hazard. However, in the context of ecological risk assessment, risk has a probabilistic meaning (EPA, 1992; 1998; Suter, 2007; NRC, 2009; Van den Brink et al., 2016)**.** For the purposes of this report, the committee adopts the probabilistic definition of risk:

> *Risk is the probability of an effect on a specific endpoint or set of endpoints due to a specific stressor or set of stressors*.

In this probabilistic definition, the *stressor* is any agent or actor with the potential to alter a component of the ecosystem. The *effect* refers to potential beneficial and harmful outcomes. And, an *endpoint* is a societal, human health, or environmental value that is to be managed or protected. Endpoints reflect decisions

that need to be made, and are sometimes determined by regulatory requirements. In the context of this chapter, endpoints include an *ecological entity* (a species, population, habitat, or ecosystem characteristic or function) and an *attribute* (a measurable characteristic of the entity)[1]. For example, endangered species of the Hawaiian honeycreeper (Case Study 3; see Chapter 3) have specific federal protections in regard to the size of their population and their habitat. In this Case Study, the gene-drive modified mosquito (*Culex quinquefasciatus*) that is unable to carry the malaria parasite will be introduced into the environment to reduce the incidence of avian malaria and protect the honeycreeper. The *stressor* in this scenario is the gene-drive modified mosquito; the *effect* is the replacement of wild-type mosquitoes with the gene-drive modified mosquito; and the *endpoint* is reducing the number of birds that die from avian malaria (see Table 6-1 for additional examples). The honeycreeper is the *entity* to be protected, and the increase in size of the Honeycreeper populations could be the measurable *attribute*.

The ability to calculate risk depends on a number of factors. First is the mathematical description of the relationships between the stressor, the environment, and the endpoint. These relationships include the distribution of the stressor in the environment, the range of probabilities that the endpoint will be exposed to the stressor, and how the stressor and the endpoint interact, including the variability in the interactions, and environmental influences on the size and distribution of changes to the endpoint.

The probabilistic definition of risk accounts for four elements:

1. Probability, reflected in the probability distributions that describe the occurrence of the stressor and the resulting effects.
2. Cultural values, reflected in the selected endpoints (thus a risk assessment may not encompass all possible effects that a stressor may produce in an ecosystem).
3. Public engagement as a mechanism to identify and incorporate cultural values of communities, stakeholders, or other publics.
4. Uncertainty, because the variability of the environmental systems, the gaps in knowledge about how these systems interact, and the challenges of accurately defining and communicating cultural values and social norms.

TABLE 6-1 Definitions and Examples of Risk and Related Terminology

	Risk *probability of an effect on a specific endpoint due to a specific stressor*	**Stressor** *any agent or actor with the potential to alter a component of the ecosystem*	**Effect** *potential beneficial or harmful outcome*	**Endpoint** *Valued characteristic of society, human health, or the environment important to decision-making*
Case Study 1 *Aedes* mosquitoes & dengue	*Probability that gene drive modified Aedes mosquitoes will decrease new dengue infections in children by 50%*	Persistence of gene drive modified mosquito in the environment	Hybridization of gene drive modified mosquito with other species	Decrease in incidence of new cases of human dengue infections in children
Case Study 5 Knapweed & biodiversity	*Probability that gene drive modified knapweed will increase population of native plants in rangelands*	Dispersal of gene drive modified knapweed	Density of wild-type knapweed	Increase in populations of native plants

[1]Environmental Protection Agency. *Terminology Services*. Accessed April 29, 2016. https://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/home.do

Given these elements, it is important for risk to be placed in a cultural framework for decision-making. In many cases, cultural values are reflected in regulations that govern the decision-making process. For example, an ecological risk assessment of a fish farm will be informed by requirements of the Clean Water Act regarding the concentration of chemicals or bacteria in the water and runoff, the size of the fishery, and the concentration of mercury in the fish. Local jurisdictions may also impost other requirements, rules to protect the community from flooding and to preserve local parks, roadways, or historical sites. These regulations reflect cultural values such as citizens' right to clean water or protected space for their homes. In the case of the Honeycreeper, a community might value the bird as its own entity while other stakeholders may value tourism related to bird watching. Both of these values could factor into the goal to reduce the burden of avian malaria on bird populations. Cultural values and preferences can be expressed as a series of criteria for the state of the system under management. Given adequate criteria, it is possible to express cultural values mathematically in the definition of endpoints.

ASSESSING ENVIRONMENTAL IMPACTS VERSUS ASSESSING RISKS

Environmental Assessment and Environmental Impact Statements Under the National Environmental Protection Act

In the United States, gene drive research will most likely be regulated under the Coordinated Framework for the Regulation of Biotechnology which assigns the primary oversight responsibilities for biotechnologies to the Environmental Protection Agency (EPA; pesticides), the Food and Drug Administration (FDA; animal drugs), and the US Department of Agriculture (USDA; plant pests) (see Chapter 8). To assess potential impacts of biotechnology, the agencies under the Coordinated framework must abide by the National Environmental Policy Act (NEPA; CEQ N Regulations, 40 C.F.R. § 1508.9; Box 6-1). Although NEPA has many strengths, it does not require a probabilistic assessment of potential risks. Ecological risk assessment, which is not currently required under NEPA but is used in several other regulatory frameworks, represents a more robust and appropriate framework for assessing the potential ecological harms and benefits of gene-drive modified organisms.

Processes Under the National Environmental Policy Act

Under NEPA, the two established processes for assessing impact as a component of formal decision-making are environmental assessment (EA) and an environmental impact statement (EIS; See Box 6-1). An *environmental assessment* is a determination of whether a federal government decision to allow the introduction (field test of environmental release) of a specific biotechnology or related product has the potential to cause significant environmental effects.

EAs generally include a wide range of scientific evidence, but they do not require quantitative or probabilistic estimates of potential environmental effects. An environmental assessment is a detailed accounting of data sources, life history characteristics, and ecological information. Although EAs contain a qualitative description of uncertainty in these datasets, they do not describe quantitatively the probability of potential effects or include a quantitative uncertainty analysis. An example of an EA with some relevance to gene drives is the "Draft Environmental Assessment for Investigational Use of *Aedes aegypti* OX513A" (Oxitec, 2016) that Oxitec submitted to FDA, as part of the company's request for approval of field trials of genetically engineered mosquitoes. The draft assessment includes a section on environmental risk assessment that presents a qualitative estimate of the risk of the release of the organism in Key Haven (Monroe County), Florida, concluding that toxic or allergic effects on either animals or humans were negligible and that the effects on the ecosystem would also be negligible.

BOX 6-1 The National Environmental Policy Act (NEPA)

Enacted in 1969, NEPA was one of the first regulatory policies in the United States to protect the environment nationwide. The NEPA process is triggered when a federal agency proposes to take a major action, such as building an airport or removing a dam. NEPA requires that federal agencies determine whether an environmental analysis is needed for a proposed action, and assess impacts of those actions that have the potential to harm the environment. Three levels of analysis are required:

1. Categorical Exclusion – a proposed federal action does not have a significant effect on the environment
2. Environmental Assessment/Finding of No Significant Impact – a proposed federal action has the potential to cause significant environmental effects
3. Environmental Impact Statement – a proposed federal action is determined to significantly affect environmental quality

The institution or agency that is initiating the action is responsible for preparing the EA or EIS. A review of the EA or EIS is conducted by the federal agency with regulatory jurisdiction over the action. NEPA allows for federal agencies to create their own procedures for meeting the requirements for an EA or EIS.

Source: Summarized from website "National Environmental Policy Act Review Process": https://www.gov/n/national-environmental-policy-act-review-process.

An EIS is required only if an EA determines that a proposed action will have a significant harmful impact on the environment. An EIS is generally a compendium of information on the environmental, economic, and other societal implications of the proposed activity. Like an environmental assessment, an EIS is not required to incorporate a quantitative, probabilistic analysis of the potential effects. However, an EIS includes alternative actions, including doing nothing, to permit comparative analysis of environmental and other implications across the different choices. An EIS often provides a comprehensive compilation of information about a proposed activity, including lists of stakeholders, cultural considerations, the regulatory landscape, and comments from interested citizens.

Some of the key strengths of NEPA process are that it is a standard approach required by legislation, supports the collection of large amounts of information about a proposed activity, it has clear reporting requirements, and includes provisions for public input. The NEPA process is also widely recognized by the stakeholder community. The disadvantage of the NEPA process, however, is that it is a regulatory process and not a decision science approach. Neither an EA nor an EIS requires a clear formulation of the problem that provides a quantitative cause-effect model. Analyses conducted as part of the NEPA process are not required to be probabilistic or report quantitatively on uncertainty. These gaps would make it very difficult to create testable hypothesis to conduct further research on gene-drive modified organisms and inform decision making.

The Process of Ecological Risk Assessment

Risk assessment is a process in which evidence on the probability of effects is collected, evaluated, and interpreted to estimate the probability of the sum total of effects (EPA, 1984). Risk assessment methodologies, which describe pertinent probability distributions and clearly identify critical uncertainties, are derived from many science disciplines, including decision sciences, psychology, statistics, mathematical modeling, and biomedicine. *Ecological risk assessment* is a related scientific process that focuses on evaluating ecological effects of exposure to one more stressors, such as invasive species, changes in land use, and infectious disease (EPA, 1992). Ecological risk assessment can be used to assess the probability

of both harmful and beneficial effects. Ecological risk assessment is quantitative, deals extensively with uncertainty, and is flexible enough to evaluate processes at large spatial and temporal scales (Van den Brink et al. 2016).

Although the field of ecological risk assessment began in the late 1980s, it is not as familiar to research stakeholders or lay publics as the NEPA process (see Appendix E for a brief history of ecological risk assessment in the United States). Ecological risk assessments are not a regulatory requirement under NEPA. However, the EPA conducts ecological risk assessments under other circumstances; for example, when evaluating the potential effects of pesticides on the environment or on endangered species. Examples of regulations that describe and require ecological risk assessment processes include the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the Resource Conservation and Recovery Act (RCRA), and the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), more commonly called Superfund, and to Toxic Substances Control Act (TSCA).

In 1998, EPA issued guidelines for risk assessors and risk managers to "improve the quality and consistency" of the ecological risk assessment process (EPA, 1998). While the guidelines include approaches to assess the risks from multiple stressors and endpoints, the focus is on the risks to populations and ecosystems from toxic chemicals (Dearfield et al. 2005). In these guidelines, the ecological risk assessment begins with a planning and scoping process, which encourages risk assessors, risk managers, and stakeholders to discuss purpose, scope, and technical approaches before the risk assessment process begins (EPA, 1998, Dearfield et al. 2005). The risk assessment process itself is carried out in three phases: problem formulation, analysis, and risk characterization. *Problem formation* is an information-gathering phase in order to define an endpoint and an ecological entity that needs to be protected (EPA, 1998). The ecological entities to be protected are typically derived from environmental protection statutes, regulations. The *analysis* phase includes two key elements: characterization of effects and characterization of exposure, which provide the data needed to predict an entity's response to the expose. The *risk characterization* phase is when results of the analysis are used to estimate risk.

Since 1998, EPA has published other documents to update the approach to selecting endpoints and the estimation of uncertainty, and an update to incorporate ecosystem services into ecological risk assessment. In an effort to design processes specific to the needs of individual programs, there is now separate guidance available for ecological risk assessments done under FIFRA, RCRA, CERCLA, and TSCA. Despite these updates, however, EPA's guidance for ecological risk assessment lags behind advances in the field.

A critical component in ecological risk assessment (and all risk assessments) is adequately taking into account *uncertainty*. Regan et al. (2002) describe two major categories of uncertainty: epistemic uncertainty and linguistic uncertainty. *Epistemic uncertainty* arises from a lack of knowledge about determinate facts. Epistemic uncertainty in risk assessments can arise out of variation in sampling results, variation in the quantitative relationship between an exposure and a response, and limitations in the models to describe cause and effect. Epistemic uncertainty is difficult to estimate without field data.

Linguistic uncertainty involves ambiguities in the terminology used to describe concepts such as species diversity, ecosystem health, or even "precise" or "accurate". For example, the term 'ecosystem health' is an example of linguistic uncertainty because an ecosystem's "health" is a normative claim regarding a characteristic (health) that it is not an inherent property of the system, but rather the meaning draws on an often unspecified value system. Minimizing linguistic uncertainty is vital in setting specifications for endpoints and communicating the results of the risk assessment to decision makers.

KEY CONSIDERATIONS FOR ECOLOGICAL RISK ASSESSMENTS OF GENE-DRIVE MODIFIED ORGANISMS

As of May 2016, no ecological risk assessments have been published for the field testing or environmental release of a gene-drive modified organism into the environment.

The 1998 EPA guidelines emphasize that a planning and scoping process should be the first step of the ecological risk assessment process (EPA, 1998). A key consideration to be discussed during the planning process for the ecological risk assessment of a gene-drive modified organism is that despite the near half century history of work, gene drive research is still at a preliminary proof-of-concept stage. For example, there are limited proof-of-concepts for gene-drive modified mosquitoes that could be used either to suppress wild-type populations (Hammond et al., 2016) or to disable their ability to carry the malaria parasite (Gantz et al. 2015). Research is underway on a gene-drive modified mouse (Campbell et al. 2015), but a proof-of-concept has not yet been published.

Many questions still remain about the efficacy and safety of gene-drive technologies (see Chapters 2 through 4). Even when research for one proposed use of a gene-drive modified organisms advances, additional research, from the molecular to ecosystem level, will still need to be conducted for other proposed uses of other organisms. What is the probability that a gene-drive construct will spread as intended throughout an island population of invasive rodents? What is the likelihood that a population of endangered Honeycreeper birds will recover if the release of a gene-drive modified mosquito reduces or eliminates the spread of avian malaria? What is the probability that gene-drive modified pigweed, *Amaranthus palmeri*, will spread to a related, non-target plant species used for food? What are the quantitative tradeoffs between pest management approaches using gene-drive modified organisms and management approaches using other methods of genetic engineering?

A third consideration is that, for some proposed applications of gene-drive modified organisms there are other strategies to address the issue. For example, there are alternative approaches to suppression of mosquito populations that could potentially be assessed as management options in a risk assessment. It may also be that a combination of a gene drive and conventional methodologies would be more effective, and at lower risk, than either approach alone—another possible consideration during planning and scoping the ecological risk assessment process.

Other key considerations about gene-drive modified organisms that will need to be accounted for in risk calculations include how the modified genetic elements move into populations, the efficiency with which the pass down from each generation to the next, and whether they are designed to affect population dynamics. Sexual reproduction between the gene-drive modified organism and the wild-type organism of the same species is required for the modified genetic element to spread in the environment, just as sharing habitat is necessary for the transmission of disease. The mere presence of the modified genetic element in other species could be considered an endpoint, for example, in risk assessment of a potential field trial on the dispersal of gene-drive modified organisms into a confined environment. Because the goal of a gene-drive modified organism is to spread, and possibly persist, in the environment, the necessary ecological risk assessment is more similar to that used for invasive species, than for environmental assessments of genetically engineered organisms.

Ecological risk assessment is equipped for the analysis of information currently available on the genetics, ecology, and potential effects of a gene-drive modified organism, and the organism's discussed complex interactions with other species and the environment. Because of the quantitative nature of the science of ecological risk assessment, it can also be used to identify uncertainties and the additional research (data) that is needed, and can inform the development of testable hypotheses in gene drive research. In consideration of the phased testing pathway (see Figure 5-1 in Chapter 5), ecological risk assessment could also be used to inform decisions about when gene drive research should move from laboratory studies (Phase 1) to field trials (Phase 2). And similarly, it could also indicate when it would be appropriate to move from field trials (Phase 2) to staged, open releases into the environment (Phase 3). However, it is not yet clear how the values of different communities or cultures will affect the selection of endpoints or how the importance of the spread of these organisms or their sequences will be considered. The considerations described here, and others, will likely increase uncertainty in the risk assessment until more laboratory and field data are available.

What might an ecological risk assessment look like for a field test or environmental release of a gene-drive modified organism? Although the overall framework of ecological risk assessment is useful in the context of gene drives, gene-drive modified organisms have important distinguishing features that

necessitate analytical tools not typically part used in conventional methods of assessing risk. Three distinguishing features are: (1) a gene drive is passed on from one generation to the next at a rate greater than that described by Mendelian inheritance; (2) a gene-drive construct can have effects on other parts of the organism's genome beyond the target; and; (3) gene-drive modified organisms are designed to spread, along with their effects, into the larger environment. The proposed uses of gene-drive modified organisms, by definition will be part of a system with multiple stressors and multiple interactions affecting multiple species and a number of endpoints. Because gene drives are intended to spread, gene-drive modified organisms will interact with a variety of species and they may even pass the gene-drive construct to closely related individuals. The physical and ecological structure of the landscape, including the distribution of habitats and human land uses as well as elements such as predators and chemical contaminants, will influence the spread of the gene-drive modified organism. In some instances multiple releases of the gene-drive modified organism may be required to achieve the desired result. The release of reversal drives has been proposed to mitigate the unintended negative impacts of gene drives on the environment; these reversal drive constructs may also introduce their own sets of wider ecological effects.

EPA's current framework and guidance documents for ecological risk assessment do not adequately address the assessment of multiple stressors and multiple endpoints. These standards and guidelines were based on risk assessments for single chemical stressors and their effects on a limited set of endpoints. The difficulty of incorporating multiple stressors into a cumulative risk assessment using these current methodologies was previously noted in the 2009 National Research Council report *Science and Decisions: Advancing Risk Assessment*. The inability of EPA's framework to deal with multiple stressors combined with multiple endpoints was a driver for the development of the original relative risk model (RRM; Wiegers and Landis, 1997; Wiegers et al., 1998; Hayes and Landis, 2004).

The committee reviewed several frameworks proposed for the risk assessment of genetically modified organisms (see Appendix C). Wolt et al. (2010), for example, proposed a problem formulation process closely related to that used for pesticides under FIFRA. The methodology is built on the premise that genetically modified crops are the stressor and that they will be limited to agricultural sites. These assumed circumstances are similar to the one chemical-one environment basis of EPA's original formulations and do not reflect the circumstances expected for many gene-drive modified organisms. In another assessment, Romeis et al. (2013) concluded that "despite the complexity of ecological systems, ecological risk assessments for genetically engineered crops do not have to be complex; they may follow the simple models used successfully for conventional chemical pesticides and biological control agents." However, the models based on the EPA's 1998 guidelines were not designed to account for the unique features of gene-drive modified organisms.

Van den Brink et al. (2016) provides a number of recommendations and specifications for performing ecological risk assessments in landscape-scale scenarios with multiple stressors and multiple endpoints (see Appendix E). Specifications that would likely benefit ecological risk assessments for gene-drive modified organisms include the following:

- Build a digital map of the study site that includes land use, topography, and the locations of sources, stressors, habitats, and endpoints.
- Map out regions in the landscape that have similar land uses, stressors, and management goals.
- Establish *a priori* the cultural values and protection goals that will determine the success of the assessment and decision-making process.
- Determine the interactions among the species and the ecological processes and functions that will be affected by the stressors.
- Construct a conceptual model that reflects the sources of stressors, the habitats, the expected effects and the impacts to the system under investigation.
- Use the conceptual model to organize all of the information that will inform the cause-effect modeling.

- Transform the cause-effect model into a quantitative structure using approaches that incorporate the dual deterministic and probabilistic nature of ecosystems.

A CONCEPTUAL CAUSE-EFFECT MODEL

An essential component of the ecological risk assessment process is developing a model that accurately portrays the relationship between stressors and endpoints, known as a cause-effect model. Cause-effect models provide a framework, based on available evidence, upon which risk calculations are built. Although much of the discussion that follows the specifications for ecological risk assessment outlined in Van den Brink et al. (2016), the cause and effect models presented here are meant to be illustrative, not prescriptive, for future efforts to conduct ecological risk assessment on gene-drive modified organisms.

Developing a cause-effect model involves three primary, interrelated steps: (1) identify a clear set of risk management questions that will be informed by the ecological risk assessment; (2) develop a detailed map of the area in question (for example, a confined field test site); and (3) construct the model and risk calculation framework.

First, identifying a clear set of management question is critical for determining the endpoints to be used in the assessment. The choice of risk management questions is heavily influenced by the relevant governance structure and publics. In many instances, the management questions are bounded by the regulations and oversight mechanisms. However, local communities and other stakeholders are critical to determining the valued components of the ecosystem in question, their relevance to human interests and well-being, and to setting risk management priorities.

Second, a detailed map of the area in question (e.g. an ecosystem or a field test site) helps to set priorities and goals for risk management. This mapping step can be summarized as "what do you care about and where is it. Maps include a variety of place-based features that may affect endpoints such as sources of exposures, location of stressors, habitats, and differences in land use (e.g. residential, commercial, and agricultural). Maps are also useful for determining how widespread a habitat is in the area of interest, or whether particular organisms of interest are clustered within the landscape. Maps help identify features that may affect, for example, the dispersal of a gene-drive modified organism, and account for them in the risk calculation. Finally, a cause-effect model and calculation framework can be developed once the management questions and the map are set. Figure 6-1 illustrates the basic format of a cause-effect model for ecological risk assessment. A conceptual cause-effect model for the ecological risk assessment of a gene-drive modified organism is illustrated in Figure 6-2. The format of these cause-effect models is based upon frameworks originally developed for nonindigenous species (Landis, 2003) to include multiple stressors and multiple endpoints, and subsequently applied to other ecological contexts around the world. For example, the fundamental methodology has been used to assess the effects of contaminants, invasive species (Landis and Wiegers, 2005), forestry management practices at large spatial scales (Anderson and Landis, 2012; Ayre and Landis, 2012), and to develop conservation priorities for the tropical rivers in Northern Australia (Bartolo et al., 2012).

The cause-effect model includes five interconnected nodes: source, stressor, habitat, effects, and impacts. The *source* is the location of the stressor and conditions of release (i.e., the mechanism, timing, and frequency of release). The source of a gene-drive modified organism, for example, depends on whether release is part of a confined field study, part of a national control program, or perhaps due to escape caused by a failure in containment. There could be multiple release sites of the gene-drive modified organism, to account for the distribution of existing wild-type organisms in the landscape. Assuming the gene drive persists in the environment, the environment itself can be considered an additional source after the initial release.

In the context of a gene-drive modified organism, the *stressor(s)* can be defined by multiple factors, including the modified genetic element, the ability of the gene-drive to propagate in the face of selection pressure, and the rate at which the genetic element is inherited from generation to generation. Unlike chemicals or invasive species, the ecological risk assessment of a gene-drive modified organism depends on the modified genotype in the organism and the efficiency with which the spreads to a specific wild target.

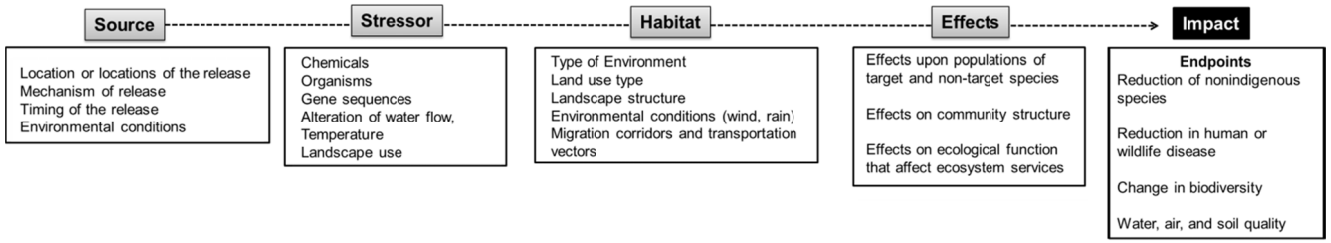

FIGURE 6-1 Basic Structure of a Cause-Effect Model. The model includes five main nodes: source, stressor, habitat, effect, and impact. Examples are listed for each one. The model requires a list of stressors and their relationship to the sources. Habitat encompasses both how the stressors occur, and how and where there they interact with organisms or other dimensions of the environment. Effects indicate how the stressor impacts the various aspects of the habitat that will alter the state of the endpoint. Impacts are the endpoints of interest.

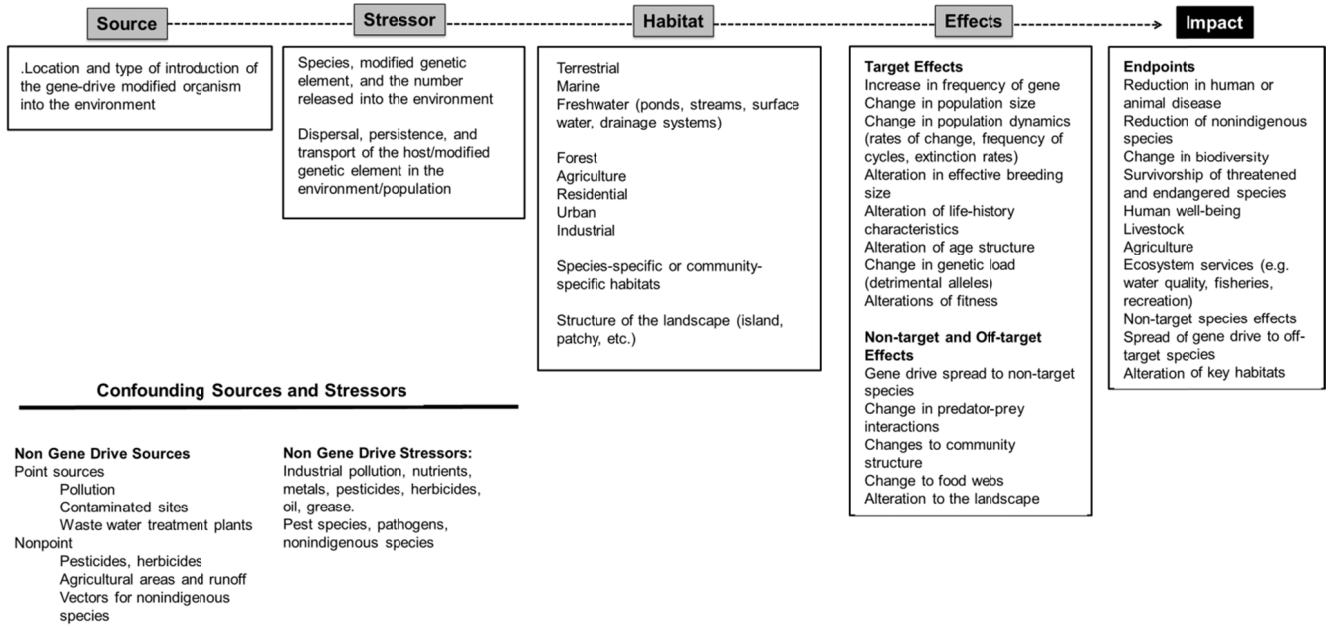

FIGURE 6-2 Generalized conceptual model for the release of a gene-drive modified organism. This generalized, hypothetical model shows examples of sources, stressors, habitats, effects, and impacts that might be involved in the release of a gene drive modified organisms into the environment. The provided examples are based in part on Landis (2004) and other examples of risk assessments performed for invasive species and forestry management (Ayre and Landis, 2012).

In common with other stressors, there will be a focus on the survivability of the gene-drive modified organism in the wild, its transport to the sites of interest, and its persistence in the environment. There also will be numerous ecological stressors, some anthropogenic and some natural to be considered. A number of other organisms and ongoing ecological processes may alter the survival of the gene-drive modified organism, the targeted wild-type organisms, and the other organisms in the receiving environment.

The range of *habitat(s)* to be evaluated could potentially be as broad for the release of gene-drive modified organisms as those considered for invasive species. A number of locations and characteristics of the environment must be considered. If the gene-drive modified organism is released to reduce the number of vector organisms, then the breeding and feeding grounds need to be included. If an invasive species is being controlled, then the habitats of the target need to be included. The terrain of the landscape and the distributions of land uses and habitats will alter the exposure and, in part, govern the effects of the gene-drive modified organism and the other stressors in the environment.

Effects will largely depend on the nature of the gene drive, and will likely include changes in population sizes, predator-prey interactions, species diversity, vector densities, among other possibilities. In some instances, a gene-drive modified organism may be used to intentionally alter the composition of an ecosystem, such as by eliminating an invasive species, which is likely to change the composition of the community and energy and nutrient flows throughout the ecosystem.

The last node, *impact*, is the endpoints of interest. Some proposed uses of gene-drive modified organisms include reducing in the spread of human disease, controlling invasive species, and preserving endangered species. Other uses are likely been proposed as the science advances. Endpoints are shaped by human values and so will need to be derived by careful and deliberate processes of public engagement and governance. Endpoints will likely vary in location and be distributed unevenly in the receiving environment. Where endpoints may move around or vary in location, cause-effect models must reflect those spatial distributions and changes, such as in the protection of the smallmouth bass, which may move into different parts of a river system depending upon water temperature, food sources, and the need to spawn.

Another important dimension of the cause-effect model is a listing of confounding sources and stressors (see Figure 6-2). Confounding factors may have significant influence on estimates of risk. For example, the use of insecticides could potentially reduce or eliminate gene-drive modified insects and thus affect the potential for the modified elements to spread as intended. Farming practices, urbanization, or other alterations to the landscape may limit the ability of gene-drive modified organisms to spread or persist in the environment. Such confounding factors will need to be incorporated into the cause-effect model.

At the end of this process, the conceptual cause-model has been bracketed by the source of the stressor and the management goals, the endpoints, and the spatial relationships in the management area.

Building the Calculation Framework

The source—stressor—habitat—effect—impact structure of the RRM can be expressed as a Bayesian network[2]. Marcot and colleagues have demonstrated the utility of Bayesian networks in conservation biology and have been pioneers in developing guidance for their use (Marcot et al., 2006; Marcot, 2012; Nyberg et al., 2006). The RRM has been modified recently to use Bayesian networks as a framework for computation and to incorporate a broad variety of evidence into the calculation of risk (Ayre and Landis, 2012; Ayre et al., 2014; Herring et al., 2015; Hines and Landis, 2014). The advantages of using Bayesian networks in ecological risk assessment have been demonstrated by Hart and Pollino (2008), Pollino et al. (2007), and Bayliss et al. (2012). Bayesian networks inherently incorporate cause-effect relationships and uncertainty and can use combinations of expert knowledge and available data (Uusitalo, 2007). Because the nodes (such as habitats and effects) in the cause-effect models are dynamic, statistical methods that

[2]Graphically depicted web of nodes that link cause and effect relationships using conditional probability to describe the interactions and to generate the probable outcome or outcomes. (Marcot et al. 2006)

account for variation in these nodes will be needed. Monte Carlo methods[3] are an approach to incorporate the probability of multiple "what-if" scenarios based on those variations into an ecological risk assessment framework (EPA 1994; See Chapter 5 of Suter, 2007). This approach generates multiple estimates of risk and thus a more complete set of information for decision-makers (EPA, 1994). For example, Hayes et al. (2015) completed a hypothetical ecological risk assessment of a modified sterile male mosquito. The authors relied upon fault tree models because experimental and field data are not yet available. The statistical analysis relies upon Monte Carlo approach to address the exposure and effects combinations (See additional discussion in Appendix E).

ILLUSTRATING A CONCEPTUAL CAUSE-EFFECT MODELS USING TWO CASE STUDIES

This section describes two hypothetical examples of ecological risk assessments on how gene-drive modified organisms might be used. The first example (Case Study 1) examines the release of gene-drive modified mosquitoes to reduce the spread of dengue to humans. In this case, the goal would be to increase the proportion of the mosquito population that does not transmit disease. The second example (Case Study 4) examines the introduction of a gene-drive modified mouse for the reduction of an invasive wild mouse population that is threatening protected marine bird rookeries.

Control of Human Dengue (Case Study 1)

The Case Study on control of human dengue includes two different scenarios (See Chapter 3). First is the release of sterile male *Aedes aegypti* mosquitoes developed using a gene drive technique. In this case the goal is population suppression, but mosquito populations could be re-established by dispersal into habitats where mosquito populations are reduced. The second is release of gene-drive modified *Aedes aegypti* that are incompetent hosts of the dengue virus. In this instance, the population of *Aedes aegypti* would not necessarily decline, but the gene-drive modified immunity to the dengue virus would ideally spread to other populations of *Aedes aegypti* by dispersal. The risk assessment process outlined here would likely be applicable to other infectious diseases of concern to humans, livestock, crops, and endangered species.

Figure 6-3 describes some of the factors to consider as part of the cause-effect pathway for the two dengue control scenarios. Such a cause-effect pathway could inform the conceptual model and eventually the probabilistic model for estimating the ecological risks of an environmental release of a gene-drive modified *Aedes aegypti* mosquitoes.

The spatial scale of the mosquito release will be a critical factor. Because *Aedes aegypti* feeds, breeds, and develops in the same areas as humans, the environment for open release would likely be an urban area with high human population densities, though the mosquito can also breed in similar environments (i.e., man-made containers for water) in rural landscapes. In the case of dengue, the assumption is that release locations would be near human habitations. The source of mosquitoes carrying the gene drive includes the location of the release, the number of insects released, and the frequency of releases. Times of introduction are assumed to correspond to time periods that reflect a unique generation (i.e., when newly-emerged females would be receptive to mating and therefore to gene transfer) and locations where breeding sites would be plentiful.

A number of characteristics are relevant to defining the stressor, the gene-drive modified *Aedes aegypti*. The genetic sequence of the mosquito suppressor drive or the sequence of the dengue anti-transmission drive is one fundamental characteristic. It is also important to consider the possibility of off-target sequences affected by the drive and their effects on survivorship and breeding.

[3]A statistical analysis that relies on repeated sampling of probability distributions of model inputs to estimate the final probability distribution for each of the model outputs (also called Monte Carlo experiments or Monte Carlo simulations) (Burmaster and Anderson 1994).

In addition to the molecular biology of the gene drive within the organism, there will be a number of other sources of stressors in the environments where gene-drive modified mosquitoes would be released. Because the habitat is likely to be an urban environment, point-source pollution from human waste materials or water-storage containers could introduce microbiota, nutrients, pesticides, agricultural chemicals, antibiotics, herbicides, insecticides, and other substances. The gene-drive modified mosquitoes may also interact with nonindigenous mosquito species, or with other genetically modified *Aedes aegypti* mosquitoes, such as those introduced as population suppressors in other research trials. Some of these organisms may require tetracycline to develop successfully into adults (as in the case of Oxitec RIDL technology). It would be important to determine whether the gene-drive modified *Aedes aegypti* is more or less sensitive to antibiotics, insecticides, or contaminants compared to wild-type *Aedes aegypti*, and to consider whether the modified mosquitos' level of sensitivity to pesticides would affect the efficacy of emergency dengue control strategies, such as chemical fogging. In addition, the rates of hybridization to related sympatric mosquito species and wild-type insects would provide an indication of the spread of the gene drive to other populations and locations. Finally, because mosquitoes that host dengue can also host other viruses, potential competition between viruses may need to be considered.

In the scenario of a gene drive that would confer an inability to spread dengue, the gene drive would need to move through the native mosquito population via breeding. As such, the rate of breeding and survival must also be estimated; any physiological or other barriers to breeding could be considered stressors in this context. Experiments to define these fitness costs would need to be performed early in the research process, such as in Phase I small-scale laboratory cage trials and Phase II larger-scale confined field experiments.

The human-centric endpoints in this case would be the decrease in the frequency of human dengue infections. This decrease should also reduce rates of morbidity and mortality associated with infection within the local human population. Since *Aedes aegypti* is a vector for diseases of cattle and other species, it would be expected that rates of mortality in these species would also decrease.

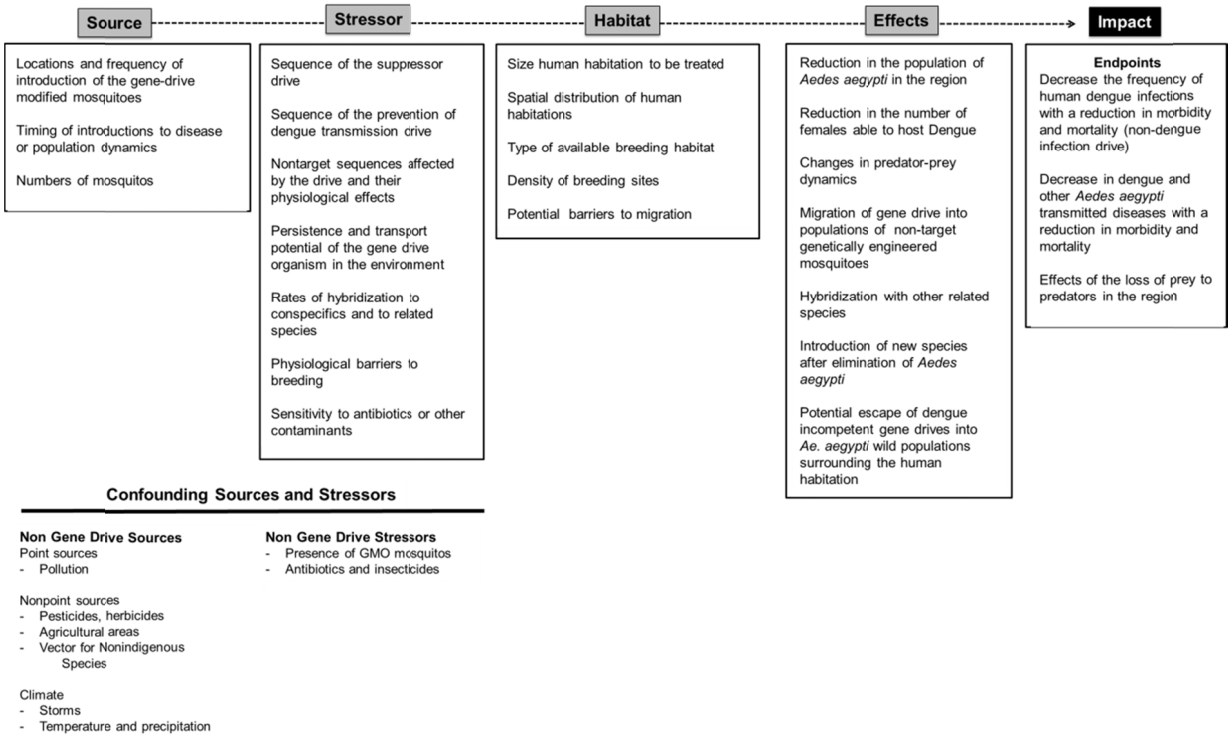

FIGURE 6-3 Conceptual Cause-Effect Model for Case Study 1.

Lastly, the elimination of a common species might alter the niche space for other insects or organisms in the region, creating a confounding stressor that could affect the outcomes. For example, wild-type *Aedes aegypti* from surrounding habitat areas (such as neighborhoods where releases have not taken place) may disperse into the gene-drive modified organism's environment. Alternatively, another mosquito species, such as *Aedes albopictus*, might enter or expand into the niche space formerly occupied by *Aedes aegypti*. Predicting the likelihood and effects of such outcomes would require information regarding the ecology of the affected region.

Eliminating Invasive Mice from Islands (Case Study 4)

This case study involves the introduction of a gene-drive modified mouse into an island environment to suppress a non-native mouse population that is threatening protected species of marine birds (see Chapter 3). The management goal, in this case, is to improve the status of the marine bird rookery. Figure 6-4 highlights selected hypothetical elements in the cause-effect pathway for the release of a gene-drive modified mouse.

The source includes consideration of the "where, what, and when" of the introduction of the gene-drive modified mouse to the island. A quantitative estimate of the expected survival of the mice and the expectation of reproduction with the island's extant mouse population would also be needed.

Stressors in this case include the sequence and physiological effects of the gene drive and any off-target sequences, as well as factors that influence the gene drive's spread, such as the potential to hybridize with conspecifics and related species. The breeding structure of the mice should also be considered; mice and many other mammals exhibit dominance behavior in which only the dominant male and female are permitted to breed. Management and mitigation strategies should also be considered in the assessment of stressors, including methods to eliminate the drive if it is not successful in achieving the desired endpoints.

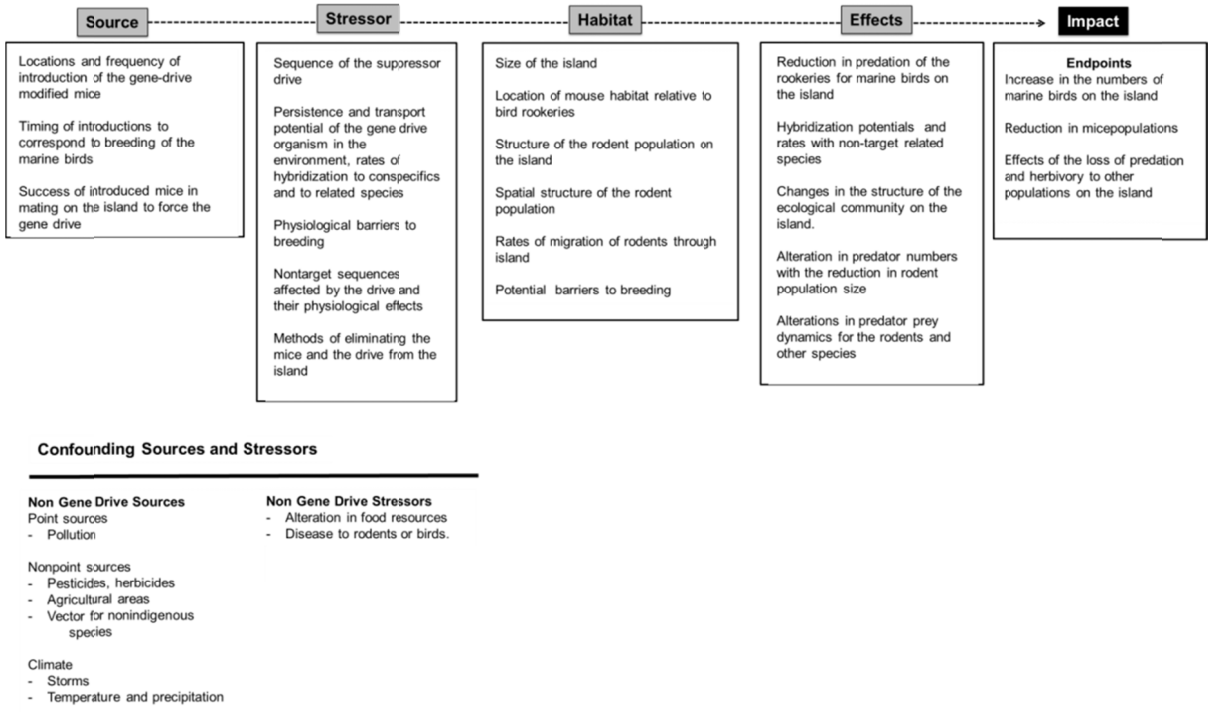

FIGURE 6-4 Conceptual Cause-Effect Model for Case Study 4.

The habitat features of the island will determine much of the interaction between the gene drive, the rodent population, and the increase in the quality of the rookery. Is there one connected or patchy metapopulation, or many sub-populations of mice on an island? What are the potential barriers to mouse breeding that would slow the rate of transmission of the gene drive? How will predators affect the population dynamics of the gene-drive modified organisms and the invasive mouse population?

Effects include the potential reduction in the rodent population along with a concordant increase in the success of the rookeries. These changes would likely have other ecological effects, such as changes to the plant and insect communities or alterations in other predator-prey interactions. The key endpoint or impact would include an increase in the number of fledgling birds.

CONCLUSIONS AND RECOMMENDATIONS

The potential for gene drives to spread throughout a population, to persist in the environment, and to cause irreversible effects on organisms and ecosystems, calls for a robust method to assess risks. Although they are widely acknowledge as valuable in other contexts, the environmental assessments and the environmental impact statements required by the National Environmental Protection Act are inappropriate tools to characterize the risks of gene- drive modified organisms. Instead, ecological risk assessment would be beneficial to gene drive research because this method can be used to estimate the probability of immediate and long-term environmental and public health harms *and* benefits.

Ecological risk assessments allows comparisons among alternative strategies, incorporates the concerns of relevant publics, and can be used to identify sources of uncertainty, making it well suited to inform research directions and support public policy decisions about emerging gene-drive technologies. This approach could potentially be built into a structured, adaptive management process to oversee the release and management of gene-drive modified organisms in the environment. As of April 2016, no ecological risk assessment has yet been conducted for a gene-drive modified organism.

> Recommendation 6-1: Researchers, regulators and other decision-makers should use ecological risk assessment to estimate the probability of immediate and long-term environmental and public health effects of gene-drive modified organisms and to inform decisions about gene drive research, policy, and applications.

Two key features of ecological risk assessments are the ability to trace cause-effect pathways and the ability to quantify the probability of specific outcomes. Both of these features are strengthened by data and models based on field trials and environmental monitoring. Reliable data and robust models are particularly crucial in situations involving multiples ecological stressors and cumulative effects, as is likely to be the case in many gene-drive applications.

> Recommendation 6-2: To strengthen future ecological risk assessment for gene-drive modified organisms, researchers should design experimental field trials to validate or improve cause-effect pathways and further refine ecological models. t

There is currently sufficient knowledge to begin constructing ecological risk assessments for some potential gene-drive modified organisms, including mosquitoes and mice. In some other cases it may be possible to extrapolate from research and risk analyses of other modified organisms and non-indigenous species. However, laboratory studies and confined field tests (or studies that mimic field tests) represent the best approaches to reduce uncertainty in an ecological risk assessment, and are likely to be of greatest use to risk assessors.

> Recommendation 6-3: To facilitate appropriate interpretation of the outcomes of an ecological risk assessment, researchers and risk assessors should collaborate early and often to design studies that will provide the information needed to evaluate risks of gene drives and reduce uncertainty to the extent possible.

In the United States, the relevant guidelines and technical documents are not yet sufficient on their own to guide ecological risk assessment of gene drive technologies, because they focus predominantly on evaluating the risks to populations or ecosystems posed by toxic chemicals, and do not yet adequately address the assessment of multiple stressors and endpoints or cumulative risk. The lack of guidance from the U.S. federal government applicable to ecological risk assessment for the gene drive research community is a critical gap.

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7

Engaging Communities, Stakeholders, and Publics

Rapidly advancing areas of research, like gene drives and their related applications, often are the subjects of multifaceted public discussion and debate. Some conversations will focus on scientific questions: Does the use of CRISPR/Cas9 to create a gene-drive modified organism cause unintended effects on the organism? How quickly will a gene drive spread throughout a population of mosquitoes or weeds or rodents? Some discussion will revolve around complex questions of ethics and values (see Chapter 4) and governance (see Chapter 8): Should gene-drive modified organisms be released into the environment? How do we decide where gene-drive modified organisms might get released? Who gets to decide? Not surprisingly, media attention to questions about gene drive research has risen sharply since the first proof-of-concept studies were demonstrated in fruit flies, yeast, and mosquitoes (see Chapter 2). Some gene drive researchers have shown an early interest in fostering broader conversations about gene drives (Esvelt et al., 2014; Oye et al., 2014), while some existing policy mechanisms, such as the National Environmental Protection Act, will require public consideration and input before a gene-drive modified organism could be released into the environment. Importantly, organized interests are likely to demand public engagement as innovation proceeds. This chapter focuses on challenges related to engagement and offers evidence-based frameworks for researchers, biotech companies, and policy makers to use to engage with public audiences about the science, ethics, and governance of gene drive research and its potential applications. We draw evidence from theoretical and empirical work in social science disciplines, including science communication, public relations, political science, psychology, sociology, and science, technology, and society , as well as the experiences of practitioners in public health to outline best practices for engagement across the diversity of potential gene drive applications and contexts.

COMMUNITIES, STAKEHOLDERS, AND PUBLICS

For the purposes of this report, we define engagement as follows:

> *Seeking and facilitating the sharing and exchange of knowledge, perspectives, and preferences between or among groups who often have differences in expertise, power, and values*.

Engagement is not just one activity (see WHO 2014, p. xxiii-xxiv) and it requires attention to multiple types of communication, deliberation, relationship building, reflection, and empowerment (e.g., Lavery et al. 2010). It is an ongoing and iterative process that does not stop at the conclusion of a research project. Neglecting engagement also undermines the important connections among values, responsible scientific practices, risk assessments, and governance.

Engaging communities, stakeholders, and publics is critical for successful decision making regarding the research, development, and potential release of gene drive technologies. These audiences for engagement exist on a continuum that relates to geographic proximity and interests (see Figure 7-1). In the context of this report, we define *communities* as groups of people who live near enough to a potential field trial or release site that they have a tangible and immediate interest in the project. While some scholarship identifies a community as "at least those individuals who share identified risks associated with the proposed research" (Lavery et al., 2010, p. 280), an emphasis on shared, identified risks leaves open the

possibility of excluding key, proximate groups, depending upon how risks are defined. While public health interventions to use gene-drive modified mosquitoes to control vector-borne diseases clearly implicate "communities" of people who live near a proposed release site, other possible gene drive projects may cast doubt on the standing of nearby communities that cannot prove a shared, identified risk. For example, if the gene-drive modified mouse in Case Study 4 were released in an area near residences on an inhabited island, the inhabitants may not constitute a community for purposes of engagement based on the definition in Lavery et al. 2010. Instead, the more common definition adopted by the committee errs on the side of inclusion. *Stakeholders* have professional or personal interests sufficient to justify engagement, but may not have geographic proximity to a potential release site for a gene drive technology. *Publics*—which we use in the plural as a reminder that public audiences are almost always subsets of the population as a whole—represent groups who lack the direct connection to a project that stakeholders and communities have but nonetheless have interests, concerns, hopes, fears, and values that can contribute to democratic decision making. Importantly, individuals may belong to more than one of these groups, suggesting that identifying who is a community member and who is "just" a member of the public will be less important than remembering that different groups may require different kinds of engagement.

MOTIVATIONS FOR ENGAGEMENT

Many factors motivate scientists, regulators, and other experts to engage with communities, stakeholders, and publics in the oversight of gene drive research. First, communities and stakeholders have knowledge that is essential to understanding the complex and variable social, political, economic, and ecological contexts in which gene drives will operate. Second, principles of justice demand both transparency in and well-informed consent to any future (experimental) trials that may affect communities of people and landscapes. Third, engagement creates opportunities for mutual learning that foster forward thinking, reflective deliberation, and the building of trust among diverse groups. Engagement thus presents challenges that must be recognized and addressed throughout the development of gene-drive modified organisms.

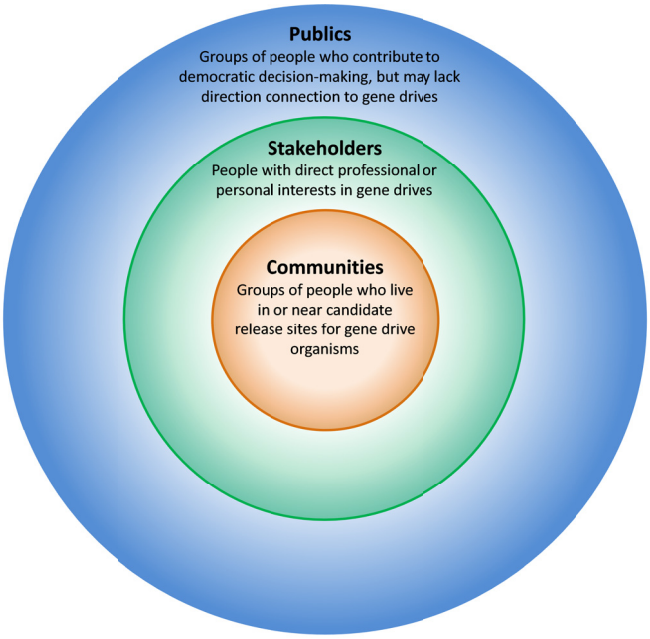

FIGURE 7-1 Communities, Publics, and Stakeholders. Audiences for engagement exist on a continuum that relates to proximity, in terms of geography and interests. People can belong to more than one group, and often do.

Integrating Practical, Experiential Knowledge

Communities have practical knowledge, insights into problems, and wisdom born of experience that may contribute pragmatically to more robust approaches to the development and governance of gene drives. Technologies exist within sociotechnical systems (Johnson and Wetmore, 2009), which means that technologies always operate in ways that connect to institutions, human beings, and social structures. For example, a gene drive for eradicating rodents on islands (Case Study 4) will not exist in a vacuum. Research and development of gene-drive modified mice will require funding from institutions, environmental release will be subject to regulatory oversight by various agencies, and diverse labor will be needed to design a release strategy and care for the rodents. Given this complexity, the "success" of gene drive technology will depend on the interaction of many parts of systems—social and technical.

Scholars of innovation have identified the importance of understanding and integrating multiple forms of knowledge—scientific, local or indigenous, and broader public preferences to the successful adoption of new ideas and technologies (Ascher et al., 2010). Recognizing the contributions of local understandings to the practice of science (Epstein, 1996; Wynne, 1996), and the ways that multiple forms of expertise interact and complement one another (Collins and Evans, 2002; Collins et al., 2004; Pielke, Jr. et al., 2007; Suryanarayanan and Kleinman, 2013) is also crucial to the success of innovation. These diverse forms of knowledge and experience are often undervalued by experts, but they are essential to a complete understanding of complex phenomena and are especially important in the context of scientific uncertainty. Put another way, technical expertise is insufficient for ensuring good governance and responsible conduct in science; making decisions when gaps in knowledge exist requires multiple forms of judgment and strong attention to values (Sarewitz, 2015).

As just one example, Chuma et al. (2010) describe how an initial distribution in Kenya of insecticide-treated bed nets to protect people from malaria-infected mosquitoes was a disappointing failure. The technology and distribution plan were sound, but the white-colored bed nets mimicked the burial shrouds used by the local population, who thus did not adopt them (Chuma et al., 2010). When new bed nets were manufactured in a different color, adoption rates—and thus the impact of the technology—increased dramatically (Gore-Langton et al., 2015). The engagement of community members in the development of the technology would have quite likely avoided this mistake. Such stories are common in the arenas of international public health and development, suggesting that gene drive technologies designed for similar purposes and contexts could be subject to the same pitfalls. In addition, the need for ongoing monitoring for the long-term success of gene drives dictates the importance of creating partnerships with local community members – who not only might conduct the monitoring but also might suggest ways to adapt standard monitoring protocols to local conditions (Lavery et al., 2010; McNaughton, 2012). This exemplifies the importance of ongoing and iterative engagement.

Democracy and Justice

Moving from pragmatic considerations that motivate the integration of multiple types of knowledge to a more normative perspective raises important questions about engagement that relate to democracy and justice. What *should* we do because it aligns with our values? The National Research Council (NRC) weighed in on this theme with particular attention to decisions about risk:

> *The normative rationale (for broad participation in risk decisions) derives from the principle that government should obtain the consent of the governed. Related to this principle is the idea that citizens have rights to participate meaningfully in public decision making and to be informed about the bases for government decisions. These ideas are embodied in laws, such as the Administrative Procedure Act and the Freedom of Information Act, although these laws and their associated procedures have not always been implemented in ways that involved meaningful participation (NRC, 1996).*

As such, engagement enhances transparency and ensures some level of meaningful participation and consent. While engagement does not guarantee an outcome that will be celebrated by all, procedures that demonstrate good-faith efforts toward respectful listening, creative compromise, and flexible practice contribute to a sense of "procedural justice." According to Ottinger (2013), "procedural justice, or the ability of people affected by decisions to participate in making them, is widely recognized as an important aspect of environmental justice" (p. 250), demanding that we foster "ongoing opportunities for communities to consent to the presence of hazards as local knowledge emerges and scientific knowledge changes" (p. 251). Regulatory frameworks may provide convening authority for engagement (see Chapter 8), but are often insufficient to achieve procedural justice. Where such laws are not in place, which may be a common context for field trials of gene drives in low-income countries, decision making processes may need to be developed to fit the political and cultural context—a lack of regulatory requirements demanding engagement does not relieve developers and scientists from the ethical obligation to engage public audiences. Examples might include *ad hoc* community meetings led by village elders or consultation with organized constituencies—furthering democratic goals regardless of the broader political context associated with the field trial's location.

Case Study 2, a gene drive in *Anopheles gambiae* to reduce the spread of malaria, for example, involves communities of people who live near the release site, and depending on the social and political infrastructure of the locale, may also involve health institutions, environmental protection frameworks, and mosquito-control agencies. Precisely because many proposed gene drives aim to solve environmental and public health problems, the severity and priority of such problems cannot be determined *a priori*, or by experts alone. Severe and high-priority problems may justify attempting solutions with the potential for negative outcomes, but such determinations must be made in contexts that go beyond technical analyses. For example, communities of people suffering from high rates of malaria may be willing to accept greater uncertainties about the safety and efficacy of a gene-drive modified organism, especially if other control measures have failed or are unavailable. Such decisions may represent cultural differences in the perception and tolerance of risk, but they may also emerge from stark differences in living conditions, public health infrastructure, and access to resources. Thus, political decision making is required, and the engagement of stakeholders, community members, and publics is consonant with democratic visions of the governance of emerging technologies.

Relatedly, gene drives (as sociotechnical systems) connect with many existing ethical and social issues, in which public audiences, stakeholders, and communities are already engaged. Examples include: the proper regulation of genetically engineered organisms in food and the environment; strategies for managing biodiversity that identify and eradicate "undesirable" species; policies related to the patenting of organisms and genetic constructs, public health practices that involve the transfer of technologies globally; and definitions of and metrics for sustainability in agriculture and other production systems. Without exception, every imagined application of gene drive technology would occur in a context already embroiled in important social debates and ethical discussions that reflect different values and priorities. Gene drives may offer new solutions that resolve the concerns of some publics; for example, as a method of eradicating rodents that has fewer non-target effects and limits animals' suffering (Case Study 4). Gene drives may also provoke new dimensions of concern; for instance, whether the combination of gene drive and patent protections lead to forms of ownership that span an entire species. The burst of media coverage surrounding advances in gene drive research (e.g., DeFrancesco, 2015; Wade, 2015; Webber et al., 2015) provide evidence of the degree to which innovation in this field connects to issues that are highly relevant to diverse stakeholders and communities.

The diversity of gene drive applications and contexts suggests that their applications may be unevenly distributed, highlighting the importance of justice considerations. For example, if a gene drive is developed to combat dengue (Case Study 1), who will benefit most from gene drive applications? Who will bear the anticipated and unanticipated negative impacts? In the context of engagement, these questions motivate attention to the voices and preferences of different communities. At present, gene drive research occurs unevenly across social and geographic landscapes, with important decisions to be made regarding which human and ecological communities may experience the first field trials of gene drive

technologies. For the foreseeable future, gene drives are envisioned to be developed predominantly in countries that already conduct gene-editing research and related product development. However, applications of gene drives will surely focus on geographies (human, political, and ecological) with less-established infrastructures. Such contrasts imply a need for engagement across such boundaries to ensure that developments are appropriate to context.

Mutual Learning

In the field of gene drive research, public engagement creates opportunities for knowledge exchange and mutual learning. For example, scientists developing a gene-drive modified mouse for release on an island (Case Study 4) will need to engage with local experts on the biodiversity, geography, and climate of the island ecosystem. Through such interactions, and especially if a field trial is designed and implemented as a partnership, significant learning would occur among the diverse experts. Irrespective of whether the trial is judged as a success or failure, all partners would be in a better position to work together effectively in the future.

As such, engagement activities are a key part of capacity building in a triple sense. First, the capacity of stakeholders and community members to understand relevant expert knowledge and partner with scientists can be enhanced. Simultaneously, technical experts increase their own capacity to understand and connect with stakeholders and community members—a skill seldom emphasized in standard training programs for scientists and engineers. Third, those who organize engagement activities build their own capacity to facilitate and organize meaningful deliberation, an especially challenging goal in political or cultural contexts in which civic engagement is not the norm. Furthermore, these interactions, if managed well, and if conducted in the absence of fundamental value conflicts, build trust among diverse groups, which creates a positive feedback loop for future engagement efforts (King et al., 2014; see Box 7-1). These views contrast sharply with the so-called knowledge-deficit model, which presumes that one-way instruction of laypersons by experts will result in public support (Sturgis and Allum et al., 2004; Bucchi and Neresini, 2008). While information is important, and learning is important to forming opinions and decision making, research shows that it is not deterministic in the way that the deficit model assumes.

BOX 7-1 Building Trust

Researchers' and government officials' efforts at public engagement are often intended to foster mutual understanding and build trust. Trust is a complex phenomenon that is essential to public interpretation of the risks of research and the effectiveness of related regulation. Although there is no universally accepted definition of trust, Hon and Grunig (1999) have described trust as having three main aspects: *confidence*, the belief that an individual or entity has the ability to do what they say they will do; *integrity*, the belief that an individual or entity is fair and just; and *dependability*, the belief that an individual or entity will do what they say they will do. Trust also depends on the available information that serves as the basis for judging these characteristics.

Discussions at the National Academy of Sciences' *Workshop on Trust and Confidence at the Interfaces of the Life Sciences and Society* (2015) emphasized that public trust in science is often tied to lay perceptions of researchers' competence and objectivity. Historically, the public has been suspicious of such technological innovations as nuclear power, vaccines, and genetically modified crops when they have doubted researchers' motivations or been anxious about misunderstanding the complex science itself. Public engagement offers researchers, funders, and governmental officials the opportunity to convey intelligible information about gene drive research, shape public perceptions regarding its credibility, and be transparent about experts' political, financial, institutional or other affiliations and conflicts that may affect public confidence in their integrity and dependability. Furthermore, engagement that embodies bi-directional exchange of information and perspectives can enhance trust by emphasizing the potential for fair and just consideration of multiple points of view.

Finally, research on deliberation suggests that engagement can foster "reflexivity" among participants, in the sense of creating opportunities for reflexive thinking to clarify one's beliefs and understandings, reflect upon and revise one's opinions, and gain insight into how different interests and values are situated in conversations about how to proceed (Dryzek et al., 2011; Dietz, 2013; Jasanoff et al., 2015). For example, stakeholders who have historically supported conservation of biodiversity and are affiliated with environmental groups that have opposed the release of genetically engineered organisms may experience tension as they confront the possibility of using a gene-drive modified mosquito to save Hawaiian bird populations (Case Study 3). Other stakeholders may have a more ambivalent initial position and perspective on gene drive applications, but engaging them may foster reflexive thinking about basic requirements of respectful and fair treatment of communities. Thus, engagement with stakeholders, experts, and community members may help clarify existing tensions that surround gene drive research and applications and offer ways forward for decision making under conditions of *value uncertainty*.

CHALLENGES OF ENGAGEMENT

While this report makes the case for stakeholder, community, and public engagement in the area of gene drive research and innovation, there are a number of important challenges and obstacles to effective engagement (Box 7-2). Many of these have been articulated by social scientists who study engagement processes empirically and with an eye toward experimenting with new formats and procedures. These insights largely come from the fields of communication, political science, sociology, and science, technology, and society. These well-documented obstacles can be addressed directly in practice through the consideration of a set of questions that can also help guide the development of efficient engagement strategies.

The first challenge is determining *who should be engaged* among the many possible experts, stakeholders, community members, and publics. Drawing such boundaries—which include and exclude certain people—and motivating their participation are not trivial tasks. While it may be obvious to engage residents of an island on which a gene-drive modified mouse may be released to protect the eggs of native birds from being eaten, less clear-cut are questions about the need to engage residents of a neighboring island or the mainland, tourists, conservation volunteers, citizens whose taxes contribute to the science foundation that makes the research possible, or individuals with moral or religious objections to modern methods of genetic engineering?

At the broadest level, scientists often speak of "public engagement," but a public audience is always just a slice or a portion of the population as a whole. Publics do not just exist; they are constructed through procedures of engagement that range from public opinion polls (with complex algorithms to achieve representative sampling, as defined by the polling agency), to public hearings (that tend to attract the most interested and organized citizens), to community meetings (that occur in particular locations and rely upon certain methods of advertisement and recruitment), to door-to-door surveys (which prioritize geography over other criteria that might define a community). Importantly, these constructions have implications not only for who has access to relevant discussions, but also the content, significance, and impacts of such engagements (Delborne et al., 2011; Delborne and Galusky et al., 2011).

Decisions about inclusion and exclusion raise a set of crucial questions that must be considered explicitly in any engagement effort (for a complementary perspective, see Kaebnick et al., 2014):

- What groups have sufficient "stake" to be considered stakeholders? Must they be impacted directly? Must they already be involved in the problem? Must they have a financial stake? Do stakeholders change with the phase of gene drive development and deployment? Do gene-drive modified organisms that are meant to spread geographically implicate ever more numerous communities?
- What knowledge or capacity is required to participate? What level of scientific literacy is expected? Who has the authority to convene an engagement activity?

BOX 7-2 Challenges of Engagement

- Who should be engaged?
- What are the goals of engagement?
- When should engagement occur?
- How can cultural differences among those involved in engagement be recognized and respected in ways that enhance deliberation?
- Should engagement lead to consensus?
- What are potential triggers for polarization?
- How should the results of engagement feed into practical and formal decision making about research and technological deployment?

- If representativeness is sought, what characteristics will be prioritized (e.g., demographic variables, political affiliations, cultural identities, interests)? What are the criteria to validate claims of legitimate representation of such characteristics?
- Do some kinds of expertise justify excluding some would-be participants? While this may appear nonsensical, deliberations including a mix of experts and laypersons can stifle the participation of those who defer to the "experts" in the room (Joss, 1998).
- How can procedural justice be established? How should conflicts of interest be managed? Does a financial stake in the technology's success (or failure) exclude someone from participating in an engagement process? What disclosure or degree of transparency of value commitments, experience, and affiliation is required?

These questions, which are by no means exhaustive, hint at the complicated decisions that precede the recruitment of actual participants for engagement. Ignoring such questions, or lacking clear answers, can lead to conflict, breakdown, or the undermining of the credibility of the engagement effort at later stages. And regrettably, despite the best efforts of all concerned, it is impossible to control for all of the factors that may affect communities and disrupt even the best planned engagement process. Given that engagement is not just one type of practice or activity, a second primary question is **what are the goals of engagement**? Answering this question relates to questions of inclusion/exclusion discussed above, but goes further to consider the relationship between procedures and outcomes. The purposes of engagement range from assessing lay knowledge about a technical issue to integrating public values into decision making. Rowe and Frewer's (2005) highly-cited typology for engagement mechanisms focuses on the desired flows of information: *public communication*, from experts to publics (e.g., outreach or educational initiatives); *public consultation*, from publics to experts (e.g., surveys or opinion polls); or *public participation*, which denotes information flowing in both directions (e.g., consensus conferences, task forces). King et al. (2014), note that there is no agreement about what community engagement contributes to the ethics of research, but that the relationships established in the course of engagement allow researchers to meet three ethical goals: (1) identifying and managing risks and benefits; (2) demonstrating respect to the community; and (3) building legitimacy for the research project.

Sophisticated procedures exist for a full range of engagement activities (Rowe and Frewer, 2005; Bucchi and Neresini, 2008; Irwin et al., 2013). This diversity of procedures serves as a reminder that engagement activities are not easily interchangeable, and each has its own limitations and challenges. For example, public opinion polls to measure the level of support for a new technology are frequently cited in political debates about governing emerging technologies. While such strategies have the advantage of accessing high levels of demographic diversity among respondents, opinion polls offer respondents little opportunity for learning and deliberation that might lead to more informed and thoughtful opinions (Sclove et al., 2010a). On the opposite extreme, consensus conferences, which do provide such opportunities, are vulnerable to critiques of a lack of representativeness (Schneider and Delborne, 2012, p. 248–49).

Even more broadly, engagement may have different meanings and significance in different contexts, although experiments in engagement suggest that deliberative forums can be successfully implemented in cultural contexts that lack such traditions (Rask et al., 2012; Rask and Worthington, 2015).

A third area of challenge emerges from the complexity of organizing people—whose behaviors are unpredictable—to discuss complicated issues—that involve a mix of facts and values—within institutional contexts that have political, economic, and cultural relevance. In other words, *doing engagement well is difficult*. Logistical challenges include:

- Obtaining adequate resources to organize the activity and incentivize participation (Kleinman et al., 2011);
- Training facilitators – who may be more likely to come with expertise in communication or social science rather than the laboratory-based skills that undergird gene drive technologies (Mansbridge et al., 2006);
- Scaling up existing models to larger national or international contexts (Cobb and Hamlett, 2008; Rask et al., 2012);
- Managing access to high quality information (Anderson et al., 2013);
- Coordinating media coverage (Schneider and Delborne, 2012);
- Balancing the benefits and drawbacks of virtual tools (Delborne et al., 2011); and
- Communicating the outputs effectively to decision makers (Delborne et al., 2013).

Research into effective community engagement strategies for the introduction of new technologies is promising, but a universal method that can be applied to the area of gene drives, or any other emerging technologies is unlikely (Guston, 1999; Kleinman et al., 2007; Nisbet et al., 2009; Philbrick et al., 2009; Sclove et al., 2010b; Rask et al., 2012; Rask and Worthington, 2015; Tomblin et al., 2015). Just as risk assessments represent a model that is highly adapted to each particular case, so also must engagement models serve as guidelines for flexible design. McNaughton has found that the range of people and issues that must be recognized, understood, and accounted for in any individual engagement process warrants long-term social research in order to develop engagement strategies that can be effectively integrated into a research program's operations (McNaughton, 2012).

Kolopack et al. (2015) conducted a qualitative case study on how community engagement activities were integrated into the day-to-day management practices of the Eliminate Dengue Program in Australia. The authors found that critical features of the Eliminate Dengue Program that contributed to meaningful engagement included funding agencies' sustained support for community engagement; core commitments and guiding values associated with community engagement, and formative social science research (Kolopak et al. 2015).

A fourth challenge is determining *when to conduct engagement*. Much attention has been given to the pitfalls of engaging public audiences late in the innovation process, which may either make the engagement irrelevant or force opinions into binary "pro" or "anti" positions. Some scholars have thus emphasized the benefits of "real-time technology assessment" (Guston and Sarewitz, 2002), "anticipatory governance" (Sarewitz et al., 2011; Guston, 2014), and upstream engagement (Wilsdon and Willis et al., 2004; Kuzma et al., 2008), which implies engagement "upstream" during the development of technology, when feedback might shape design choices made during the innovation process. In tension with this view, premature engagement with community and public audiences can present a range of other challenges. For example, in the early stages of research it is difficult, if not impossible, to predict whether experiments will lead to the development of future technologies or what the potential benefits and harms of those technologies might be (Tait, 2009; McNaughton, 2012;).

Fifth, *cultural differences between groups with different kinds of expertise* make engagement across those groups difficult. Research has shown that scientific and public audiences often have different attitudes toward technological risks (Kahan, 2012; Mielby et al., 2013; Su et al., 2015). In the case of gene drive research for the control of vector-borne diseases, cultural nuances can make engagement challenging across

all communities. Differences in knowledge, values, language, social status, and communication styles all combine to stress any attempt at engagement. Careful facilitation and thoughtful design are required to minimize the likelihood of frustration among participants or breakdown in good-faith deliberation.

Sixth, *engagement may not always lead to consensus*—especially as efforts are scaled up to include more diverse publics. When there is a lack of consensus, it can be difficult to discern whether disagreements stem from disputes about evidence or differences in values. Evidence disputes suggest that further research to reduce uncertainty may resolve disagreements among stakeholders. Yet much decision making surrounding the governance of science and technology involves an "excess of objectivity" (Sarewitz, 1996), meaning that different experts can be found to "objectively" defend various political or other stances. Furthermore, incentives exist to frame disputes over values as factual disputes (Pielke, Jr. et al., 2007). Pielke's solution is to encourage more teams of experts to operate as "honest brokers of policy alternatives," expanding and clarifying the range of policy options available to decision makers, but his framework does not make clear the available roles for communities, publics, and stakeholders with non-certified expertise. Therefore, managing participants' expectations of an engagement process will be important, particularly in regard to stakeholders' expectations that any decisions made will reflect their preferences, which is not possible where there is no consensus.

If engagement does not lead to consensus, how does one confirm that an engagement process is effective, or that a community is truly engaged? Standardized approaches and metrics to address this question are elusive and a topic of discussion (Alderman et al., 2013). However, community scorecards and other social auditing tools have been successfully used to capture public feedback and guide health priority settings in some contexts (World Bank, 2015). Similar tools could be applied to engagement processes in research with gene drives. Regardless of the approach, it is generally acknowledged that indicators of success will vary with context (Sibbald et al., 2009).

Seventh, social science research has described problems such as *polarization cascades***,** particularly when there are divisive political or ideological perspectives that can undermine engagement (Sunstein, 2009; Tait, 2001). Relatedly, *social amplification* of concerns may occur when, for example, an overemphasis on uncertainty is used as a political tool to reinforce negative or positive framings of science and technology (Stirling, 2014; Tait, 2014).

FRAMEWORKS TO GUIDE ENGAGEMENT

From these challenges, it is clear that public engagement is dynamic and context-specific. There is not a standard approach that can or should be used across all scientific research and related applications. Engagement is challenging for many scientific communities, and so lessons can be drawn from prior efforts to design and evaluate engagement (Mazerick and Rejeski, 2014; NRC, 2015; NASEM; 2016). For example, NASEM (2016), *Effective Chemistry Communication in Informal Environments,* presents a five-part communication framework "based on the best available empirical evidence from the research literature in informal learning, science communication, and chemistry education" (see Box 7-3). Each element of the framework is based on the notion that engagement and evaluation processes should be designed in advance. The framework emphasizes that targeted goals should take into consideration the interests, values, and perspectives of communities, stakeholders, or publics. Equal emphasis is also given to the need for evaluation to occur throughout the engagement process, not just at the end. Although this framework was developed to guide scientists in the design of engagement activities about chemistry, it is broadly applicable to other engagement contexts and areas of science and technology, including gene drives.

Public engagement experiences on issues related to gene drives, such as the release of genetically modified mosquitos, can also inform engagement efforts for gene drives. Lavery et al. (2010) summarized key points to consider for effective community engagement in global health research in Mexico that was developed as part of a field study involving genetically engineered mosquitoes. To ensure that community members can be active participants from the outset, Lavery et al.'s framework begins with site-selection criteria that call for the capacity to be active participants in research and engagement. It is imperative to

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characterize the community and build trust with relevant authorities early to ensure that the goals of the research are clearly articulated and that investigators are afforded the opportunity to understand the community's perceptions of and attitudes towards the research. Awareness of those persons and groups that will integrate the cultural background is also a critical component of their engagement framework; therefore, tailored practices of engagement may be required in developing countries (Tindana et al., 2007). Lastly, continual review of the outcomes of and additional need for engagement is essential to strengthening the process as the research program progresses.

BOX 7-3 Example of an Evidence-Based Framework to Guide Science Communication[a] (NASEM, 2016)

Element 1 **Set communication goals and outcomes appropriate for the target participants[a]**	• Who are my participants? • What will my participants find interesting, relevant, or engaging? • What participant-relevant goals and outcomes would I like to achieve? • What can I expect to gain from this activity?
Element 2 **Identify and familiarize yourself with your resources**	• Are there organizations I can partner with? • What physical resources are available, such as accessibility and space?
Element 3 **Design the communication activity and how it will be evaluated**	• How can I test the communication activity in advance to see whether it is suitable for my participants? • How do I relate to my participants to build trust? • What methods should I use to evaluate my activity?
Element 4 **Communicate!**	• Am I following my engagement plan? • Am I still working toward my targeted goals and outcomes? • Are the participants engaged? • What aspects seem to be of particular interest to them?
Element 5 **Assess, reflect, and follow-up**	• Have I achieved my intended goals and outcomes? • How can I apply what I learned in my communication experience to the next time?

[a]The report's authoring committee chose the term *communication* because they felt it encompasses a wide range of possible interactions with publics and that it emphasizes engagement.
[b]The report's authoring committee used the term *participant* to mean any person or group of people with whom chemists might engage.

The World Health Organization, in partnership with the Foundation for the National Institutes of Health (FNIH) and the Special Programme for Research and Training in Tropical Diseases (TDR), has also proposed a framework for community engagement specific for testing genetically modified mosquitoes (WHO, 2014). The framework gives special attention to the communities for whom the engagement is intended. For example, engagement at the community level will focus on those persons within the primary area where genetically modified mosquitoes will be released, such as a village in Africa, whereas engagement with stakeholders will focus on broader groups and organizations with environmental concerns about the release of the modified mosquitoes. The expertise required for effective engagement will therefore be unique to each level. Related to levels, the phase of testing, which dictates the scale of study, will also require investigators to identify appropriate engagement facilitators. Investigators can outline levels of engagement by phase of evaluation to guide them with identifying the appropriate stakeholders for engagement and the type of expertise that will be required in order for the effort to be effective.

CONCLUSIONS AND RECOMMENDATIONS

The committee did not attempt to prescribe a single method of engagement for gene drive applications, but rather, aimed to provide considerations for responsible practices through the following conclusions and recommendations.

Engagement with communities, stakeholders, and publics is an essential part of research on and development of emerging technologies, including gene drives. Engagement can facilitate mutual learning and shared decision-making, support democracy and justice, help identify and assess potential benefits and harms, and provide a mechanism to explore difficult-to-articulate questions, such as the human relationship to nature. Engagement is also important as a matter of respect for and empowerment of the people likely to be most closely affected by the potential use of gene-drive modified organisms. The question is not whether to engage communities, stakeholders, and publics in decisions about gene drive technologies, but how best to do so.

The outcomes of engagement may be as crucial as the scientific outcomes to decisions about whether to release of a gene-drive modified organism into the environment. Thus, engagement cannot be an afterthought; it requires effort, attention, resources, and advanced planning. Those who organize and facilitate engagement about gene drive research need to explicitly consider who is to engage with whom, along with when, how, and for what purpose the engagement will occur. If engagement efforts are meant to have impact beyond mutual learning, it will be important those goals and plans are transparent to participants.

Engagement won't happen all at once; it can and often occurs in stages and iteratively. One stage of engagement can inform the next phase of research and the next phase of engagement.

Recommendation 7-1: Research plans to develop gene drives should include a thoughtful engagement plan that considers relevant communities, stakeholders, and publics throughout the process of research, from proposal development through, if applicable, the release and monitoring of gene-drive modified organisms in the environment.

Recommendation 7-2: Because engagement can contribute to defining the values and preferences of communities, stakeholders, and publics about gene drive technologies, researchers and risk assessors should integrate engagement into the construction of risk assessment models. In turn, the outputs of risk assessments should feed back into engagement efforts.

Recommendation 7-3: Funders of gene drive research should allocate a percentage of technical research grants' budgets to engagement activities, both to encourage good practice and to advance knowledge of effective engagement techniques.

Short-term or online training of scientists is unlikely to build sufficient capacity to design and implement engagement activities without drawing upon additional expertise—especially because each engagement effort must be tailored to a specific context and purpose. Strategies will be needed to study, develop, and foster meaningful community engagement for specific research endeavors, as well as broader public engagement about the overall goals and consequences of gene drive technologies. These efforts will likely need to draw upon a wide diversity of examples and instructive scholarship, as well identify facilitators from among groups' measure of distance from the technological research and development. Such experiences will build the capacity of gene drive researchers to participate and play increasingly important roles in future engagements.

Strategies will also be needed to evaluate engagement efforts to determine if they are working as intended. Such evaluations need not overwhelm a project's financial or human resources in order to contribute meaningfully to tacit and formal knowledge about the success of engagement efforts. In addition, interdisciplinary efforts could also enable the convening of a new formal consortium on engagement on gene drive research that would communicate "lessons learned" among scholars, scientists, practitioners, stakeholders, and communities.

Recommendation 7-4: Gene drive researchers should take a multi-disciplinary approach to engagement, partnering with social scientists, ethicists, evaluators, and practitioners with expertise in engagement to develop and implement engagement plans.

Recommendation 7-5: Researchers, funders, and policy makers should develop and implement plans to evaluate engagement activities related to gene drive research. When possible, these evaluation s should be published in the scholarly literature or otherwise made available as part of a shared repository of knowledge.

Engagement is not a one-size-fits-all endeavor. Engagement strategies will need to adapt and remain sensitive to cultural, social, and political contexts. The diverse proposed environments for gene drive research and potential release suggest that attention to this principle will take time, sensitivity, and a commitment to listening and learning. It is important to recognize that engagement practices always include some members of communities, stakeholders, and publics and exclude others, and that engagement sponsors (e.g., companies, government agencies, non-governmental organizations), participants, and broader publics may have different expectations of and goals for engagement. In addition, disagreements over values, standards of evidence, or preferences for desired outcomes may remain even after fruitful deliberation. Because of these complexities, efforts to build mutual trust and maintain procedural justice will be paramount. Such efforts could include:

- Transparency from organizers about their decisions on who is or is not included in engagement and the basis on which those decisions are made.
- Open acknowledgement. from all parties of the diversity of goals that people may have and how specific procedures aim to fulfill those participants' expectations
- Open acknowledgement from all parties that successful engagement may not always—or even often—result in consensus.

Recommendation 7-6: Researchers, funders, and policy makers should adapt engagement plans that are relevant to the social, cultural, and political contexts in which gene drive research may be planned. This contextualization is especially important when the engagement process is organized or sponsored by groups and individuals whose origins and interests are different from those of the stakeholders, communities, or publics to be engaged. In such situations, particularly when field-testing or environmental release of gene-drive modified organisms are intended, it is critical to include local experts as partners in the design and implementation of the engagement process.

Recommendation 7-7: Researchers, research institutions, and other organizers should explore ways to diversify engagement activities in order to include different voices at different times, especially given the intention for some gene-drive modified organisms to spread over time and across significant distances. Early in the development process, organizers should identify critical groups and time-points for interaction; as the research unfolds, these decisions should be revisited to ensure engagement activities remain appropriate and such related decisions should be revisited as the research unfolds.

Recommendation 7-8: Researchers, research institutions, and other organizers should design engagement activities to respect different points of view. Such deliberation may enable participants to reflect upon their own beliefs and understandings in new ways. Dissent should be captured and considered carefully, but engagement does not require the dissenters to be convincing or convinced.

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8

Governing Gene Drive Research and Applications

The governance of science ensures that research, whether in a laboratory or in the field, is conducted with appropriate oversight and in accordance with societal values. Governance of technology has a similar role in regard to how the products of research and innovation enter society and the environment. Thus, the governance of science and technology concerns questions about who conducts and oversees research activities, who benefits from scientific advances, mechanisms to ensure that members of the public are protected, and mechanisms to include communities, stakeholders, and publics in making decisions about research and its applications. The accelerated pace of gene drive research, combined with the ease of use of molecular technologies to create gene drives, has prompted discussion of the capacity of existing professional and regulatory mechanisms to govern these activities. The novelty of this technology also provides an opportunity to reflect more generally on the principles governing scientific research and suggest areas for improvement.

The previous chapters of this report identify values and ethical questions reflected in and challenged by gene drive research and its related applications. Through a set of case studies we also explored ways to assess risk and principles for how and why to engage affected communities, other stakeholders, and broader publics, in discussions about gene drive research. This chapter builds upon those themes to answer two primary questions:

- What general principles could guide the evaluation and improvement of governance systems as gene drive research matures?
- Do existing governance systems in the United States and abroad adequately promote and protect public health, the environment, and other societal interests?

These questions are critical for the future of gene drive research and the potential release of gene-drive modified organisms into the environment.

WHAT IS GOVERNANCE?

The definition of governance varies by scholarly discipline, politics, and culture. Governance includes standards - *voluntary* norms and policies that arise from tradition or consensus processes that are often widely accepted, but not enforceable by law. It also includes regulation—*mandatory* policies agreed upon by legislative authorities that are enforceable by law. For the purposes of this report, the committee adopts a broad definition that is derived from the World Bank's World Wide Governance Indicators (World Bank, 2015):

> *The process of exercising oversight through regulations, standards, or customs through which individuals and communities are held accountable. This includes:*
>
> - *the process by which authorities are selected, monitored, and replaced;*

- *the capacity of governing authorities to formulate and implement sound policies; and*
- *the respect of governed communities for the authorities and processes that govern their activities*.

This definition encompasses a wide spectrum of policy tools, including norms and guidelines that stretch from traditional customs to regulation.

Governance of Science and Technology

The importance of governing science has been broadly accepted since the development of the Nuremberg Code after World War II (Annas and Grodin, 1992). The governance of science in the post-WWII United States has included federal and state legislation and other governmental regulations, professional and institutional codes of conduct for scientists, systems of professional certification and accreditation of the education of scientists and manufacturers, public engagement in discourse over science, and other mechanisms to align scientific activities with societal interests in health, environmental integrity, or other social goods (NRC, 2015).

The governance of science consists of both a set of policy tools for self-governance developed by the scientific community, and mandatory policy tools developed by entities outside the scientific community. In self-governance, the scientific community itself defines, establishes, and enforces professional codes of conduct and guidelines that define and govern best practices and unacceptable behavior. These differ from systems of public regulation, wherein national or state authorities have legal powers to oversee the processes and products of research and technology. There is a middle ground in which governments create guidelines that shape the behavior of scientists and research institutions by creating norms and expectations of good practice. Table 8-1 provides some examples of policy tools that govern scientists, research institutions, and applications of science and technology.

TABLE 8-1 Examples of Policy Tools Used to Govern Science and Technology

Policy Tool	Description	Examples
Professional Scientific Standards or Norms	Self- governing mechanisms within the scientific community	Hippocratic Oath, the Nuremberg Code; American Society of Microbiology's Code of Ethics[a]
Guidelines on the Practice of Scientific Research	Developed by recognized scientific authority	World Health Organization 2014 Guidance Framework for Testing Genetically Modified Mosquitoes
Requirements of Research Funders and Sponsors	Enacted in funding agreements rather than through formal law, and often implemented at the institutional level	U.S. National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules; Institutional Biosafety Committees; Institutional Animal Care and Use Committees
Regional-Level Regulation of Science and Technology	State or national regulation with binding legal force	California Department of Fish and Game
National-Level Regulation of Science and Technology	Governmental regulation with binding legal force	Human subjects research protections in all federally funded research (i.e., the Common Rule and related regulations)
International Agreements	Regulatory and non-regulatory agreements between countries.	International Plant Protection Convention to protect cultivated and wild plants by preventing the introduction and spread of pests.

[a]ASM, 2005.

The Spectrum of Governance for Biotechnology: From Prevention to Promotion

The regulation of biotechnology is seldom straightforward. Certain biotechnologies have been controversial precisely because there are disagreements about the levels of risk and uncertainty that they involved, as well as what uncertainty should mean for decision makers (Tait, 2014). Uncertainty attends all governance decisions about safety and hazards because the probabilities produced in risk assessment are never zero or 100% (Charo, 2015). Existing governance for biotechnology products is context dependent, and there does not have to be only one approach to the governance of all biotechnology. Governance tools often take different policy directions across national systems (Tait, 2008). Difference societies will tolerate different levels of uncertainty under different circumstances, which results in diverse stances on how to manage *innovation*, the process through which knowledge is converted into potentially useful applications. However, common stances can be organized into four general categories (Charo, 2015; Box 8-1).

Innovation and precaution can be complementary with public understanding and effective oversight creating the public confidence needed to support risk-taking and novel technologies (Baltimore et al. 2015; Carrol and Charo, 2015). Nonetheless, some have challenged whether the precautionary principle can truly be implemented (Sunstein, 2009, El-Zahabi et al., 2010). Oversight, however, must be balanced with the potential benefits of innovation. Regulatory regimes that are designed to be adaptive to the lessons of future experience and unexpected harms or benefits could enable the continued development of the science and technology with increased capacity to deliver benefits to society in the future.

KEY CONSIDERATIONS FOR GOVERNING GENE DRIVES

Gene drives have two major features that distinguish them from other types of biotechnology: they intentionally spread a genetic trait through a population, and their effects on ecosystems are potentially irreversible. These two features carry important implications for the governance of gene drive research and related applications.

First, it is the goal of using a gene drive to spread a genetic trait through a population. Intentional spread challenges current governing systems for biotechnology predicated on managing risk by containing genetically modified organisms through physical, biological, or environmental methods. A mechanism designed to spread genetic information has consequences associated with accidental release that differ from other genetically modified organisms. Unexpected gene flow is a concern that regulators of current genetically modified organisms seek to mitigate, whereas such flow is expected and even intended for organisms bearing gene-drive constructs. In addition gene-drive modified organisms are expected, at least under some conditions, to cross legal boundaries and territories. Actions with trans-border effects complicate already difficult questions of governance, e.g., who should make decisions, who should be consulted, who is accountable to whom, and how liability should be handled as a legal matter. Thus, the anticipated trans-boundary effects of gene-drive modified organisms give rise to the need for international policies or regulation that build agreements between countries.

BOX 8-1 Approaches to Governing Science and Technology

- *Promotional*: support and remove obstacles to innovation
- *Neutral or Absent*: neither promote nor hinder biotechnology
- *Precautionary*: slow advancement or introduction of biotechnology
- *Preventative*: prevent, defund, or ban certain types of biotechnology applications

Source: Modified from Charo (2015)

Second, gene drives heighten concerns about irreversibility. Once a gene-drive modified organism is released into the environment, any unintended effects on other species or ecosystems could be potentially irreversible. Thus, it will be important for governance to take into account the potential need to 1) stop the spread of a gene-drive modified organism that has been released; 2) mitigate harm and restore the environment; and 3) provide compensation for harms that cannot be addressed by mitigation or ecological restoration measures. The characteristic of biological irreversibility has important implications not only for physical-material risk, but also for the perception and communication of harms and benefits. Public perception of technological risk tends to respond to known factors that raise special concern. Technologies that are novel and less well-known, whose use is not directly perceptible, or which have delayed outside effects, also tend to be of higher public concern (Slovic, 1987).

General Principles for Governance of Gene Drives

Developing effective governance of science and technology, in general, is challenging because these frameworks must reflect the values of multiple publics, stakeholders, and communities. Some sets of values may align readily, for example, that we should combat human disease and promote and protect human well-being (see Chapter 4). Other sets of values may be in tension or conflict with one another, for example, that ecosystems should be protected and that humans should not tamper with nature (see Chapter 4). An ideal governance framework seeks to ensure that science and technology are safe for people and the environment, deliver the expected benefits, and are developed and used responsibly following high ethical standards. For instance, in some fields, a technical risk assessment of an experiment's potential harms and benefits is a foundation for decision making (Emanuel et al. 2000). Furthermore, it is clear that governance is a joint responsibility involving the collaboration of a broad range of publics—including public, private, governmental, lay, and professional individuals and organizations.

Based on the distinctive features of gene drives and the discussion of values, risk assessment, and public engagement in previous chapters, several desirable features can be identified for their governance, prior to examining whether existing mechanisms include these qualities (Box 8-2).

First, risk assessment is thorough and includes a variety of experts. As indicated in Chapter 6, robust models of risk assessment can inform decision makers at each level of governance. Risk assessment will need to be generally informed by the diverse forms of expertise that gene drive technology requires, including knowledge on best practices in laboratory and field research. Furthermore, it is important that risk assessments identify, and when possible, account for sources of uncertainty, confounders, and other limitations. The release of gene-drive modified organisms requires predicting the consequences of genetic modifications in complex environments. This is and will likely remain an imperfect task; sources of uncertainty and ignorance will need to be clear to decision makers.

BOX 8-2 Desirable Features of Governance for Gene Drives

Risk assessment is thorough includes a variety of experts, and the limitations and sources of uncertainty are well-defined

- Engagement of communities, stakeholders, and broader publics feeds into the governance process
- Authority, responsibility, and methods for accountability are clear
- The level of oversight is proportionate to the risks involved as well as sensitive to the ways that regulation can restrict innovation
- The ability to adapt in the face of scientific and social developments
- The capacity to anticipate trans-boundary movement of gene drives and prepare appropriate mechanisms for agreement and cooperation between and among countries

Second, a process to engage affected communities and broader publics feeds into the governance process. The anticipation of those affected by these decisions is a central tenet of democracy, and, public engagement processes can be useful for bridging gaps between researchers, communities, other stakeholders, and broader publics (see Chapter 7). Communication among scientists, risk assessors, and policy makers with communities has long been seen as an important component of the governance of risk—not just of decision making, but also in the characterization of risk (NRC, 1996). Effective governance creates and sustains effective mechanisms for on-going conversations with communities, especially those proximate in time and place to proposed activities, before and after decisions are made about research and technology. Applying this principle here—if and when gene drive research moves outside the laboratory, iterative communication with affected communities will be a key part of the risk assessment process. Engagement with broader publics is also essential when important new questions about science and technological governance arise, especially because gene-drive technologies are often envisioned to spread beyond the boundaries of discrete human communities.

Third, clear lines of authority and responsibility and methods for accountability are essential to good governance. Due to the distinctive forms of harms and benefits entailed in using gene drives, and the growing public interest in the technology, clear lines of authority and responsibility will be even more important, both in terms of the effects of gene drives and decision making about them. Accountability as a norm aims at generating desired performance through control and oversight, facilitating ethical behavior, and promoting democratic governance through institutional reforms (Dubnick and Frederickson, 2010).

Fourth, proportionality is another central characteristic of the effective governance of technology, with the level of oversight proportionate to the risks involved in the technology as well as sensitive to the ways that regulation can restrict innovation. It is possible, in other words, to "over regulate". Governance has an important relationship to innovation. Certain forms of governance and regulatory approaches, based on different responses to uncertainty, may adversely impact the development of important new technologies and their potential benefits to society. That being said, the protection of society's interests and values, as well as public perceptions, may require rigorous oversight in some cases. Proportionality may be especially important to seeing that a single level of oversight should not necessarily be applied across functions and across levels of integration with the environment.

Fifth, good systems of governance are adaptive in the face of scientific and social developments. In arenas of biotechnology like gene-drive modified organisms, the technological frontier will shift constantly. A rigid approach that cannot adapt to changing technological and institutional conditions will quickly become outdated and potentially harmful to the interests it was designed to protect.

Finally, the ability to anticipate trans-boundary movements of gene-drive modified organisms will be critical. Trans-boundary effects, especially harms, can give rise to complex legal and political controversies. Therefore, as a principle, the governance system will need to be conducive to multilateral approaches to governance—including mechanisms, agreements, or norms—in order to encourage cooperation across borders.

RELEVANT GOVERNANCE FRAMEWORKS FOR GENE DRIVE RESEARCH AND APPLICATIONS IN THE UNITED STATES

Biotechnology emerged in the early 1970s with the development of recombinant DNA (rDNA) technology. This new technology allowed the *movement* of genes from one organism to another to create "engineered" organisms containing genetic combinations that did not exist in nature. From the beginning, rDNA research raised concerns about the potential harms posed by such organisms. After a 1973 conference on rDNA research helped spur a National Academy of Sciences inquiry into its potential hazards (Krimsky, 1982), biologist Paul Berg assembled a team of distinguished scientists to plan what would become the 1975 Asilomar Conference on Recombinant DNA. The 1980s and early 1990s saw intense debate on the appropriate form of regulation for genetically modified organisms, leading to divergent regulatory approaches in the United States and the European Union characterized as *product based* and *process based*, respectively (Tait, 2008). Regulation based on the potential *function* of a gene drive has now

been proposed, where risk is defined as "the ability to influence any key biological component the loss of which would be sufficient to cause harm to humans or other species of interest" (Oye et al., 2014). This is essentially a product-based approach that embraces a case-by-case risk assessment of gene-drive technologies. However, the concept of function usefully underscores how important it is that regulatory assessments capture the potential harms to human and environmental health posed by the intended uses of gene drives in their social and ecological contexts.

Coordinated Framework for the Regulation of Biotechnology

In the United States, regulation of gene-drive modified organisms will most likely fall under the Coordinated Framework for the Regulation of Biotechnology (OSTP, 1992). Crafted in 1986 and updated in the 1990s, the Coordinated Framework outlines a comprehensive regulatory policy for ensuring the safety of biotechnology products based on their intended use. Regulatory authority for genetically modified organisms under the Coordinated Framework is shared across the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and the US Environmental Protection Agency (EPA). FDA has regulatory oversight over genetically-modified foods, or any modified organisms interpreted to contain an "animal drug." USDA oversees regulation of any organisms that are potential plant pests. EPA has oversight over products perceived to be pesticides. If biotechnology products have potential environmental consequences, all three agencies must adhere to National Environmental Policy Act (NEPA). A fourth agency, the Centers for Disease Control and Prevention, has regulatory authority if/when public health is threatened; for example, if a gene drive intended to prevent the spread of dengue (Case Study 1, Chapter 3), caused the Asian tiger mosquito to be a more effective transmitter of another disease, such as chikungunya.

The regulatory landscape pertinent to gene-drive technologies is itself evolving, as the U.S. system of regulating biotechnology is currently being reassessed. Pending changes stem from awareness within government, industry, and civil society that there are potential inconsistencies and gaps that require clarification and adjustment. In July 2015, the Obama administration issued a memorandum directing the "primary agencies that regulate the products of biotechnology— EPA, FDA, and USDA—to update the Coordinated Framework, develop a long-term strategy to ensure that the Federal biotechnology regulatory system is prepared for the future products of biotechnology, and commission an expert external analysis of the future landscape of biotechnology products to support this effort" (Holdren et al., 2015).

An Examination of Governance Mechanisms through a Phased-Testing Pathway

This section canvasses the national and international oversight mechanisms that are most relevant for research on gene-drive modified organisms and potential applications of the technology. The committee uses this landscape to consider the adequacy of U.S. and global capacity to protect public health and the environment from the potential harms of gene-drive modified organisms, and to identify major concerns or gaps. The governance landscape in this section is described through the lens phased testing pathway from laboratory-based research to field trials to environmental release described in Chapter 5 (See Figure 5-1). To aid the committee's analysis, Case Studies (see Chapter 3, Box 3-1) of likely gene-drive applications are used along with more hypothetical examples to discuss considerations for and gaps in governance.

Governance Mechanisms for Phase 1 (Laboratory-Based Research)

In academic settings, laboratory experiments on gene-drive technologies are overseen at the institutional level through Institutional Biosafety Committees (IBCs). These committees are the cornerstone of institutional oversight of recombinant DNA research, and are the primary oversight mechanism for research involving genetic modification at National Institutes of Health (NIH)-funded institutions. IBCs work with researchers to develop appropriate protections of health and environmental safety for experi-

ments involving biotechnology. These committees assess the risk of proposed experiments and recommend containment mechanisms based on categories of risk.

For research funded by NIH, the NIH Office of Biotechnology Activities ultimately oversees practices for the safe containment of basic research involving the creation and use of organisms and viruses containing recombinant or synthetic nucleic acid molecules. IBCs are accountable to the NIH Office of Biotechnology Activities and must implement stipulated guidelines for biosafety known as the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH 2016a). When certain kinds of novel experiments are proposed to local IBCs, these must be referred to the Office of Biotechnology Activities, and its advisory body, the Recombinant DNA Advisory Committee (RAC)(NIH 2016b), for consideration and recommendations. A 2014 Institute of Medicine report, *Oversight and Review of Clinical Gene Transfer Protocols: Assessing the Role of the Recombinant DNA Advisory Committee*, recommends that the kinds of protocols the RAC assesses should be restricted, particularly if an assessment can be adequately performed by another regulatory and oversight process such as an IBC (IOM, 2014, p 4.). However, these recommendations were developed before the first gene drive proof-of-concept studies were published, and may need to be reconsidered in light of potential gene-drive technologies. Indeed, a new National Academies of Sciences, Engineering, and Medicine study is underway that will identify near term biotechnology products, such as gene-drive technologies, and provide advice on "the scientific capabilities, tools, and expertise that may be necessary to regulate those forthcoming products."[1]

IBCs and other government policies reinforce a system of professional best practices in research. Best practices standards in research consist of both technical and ethical considerations and are essential for the research enterprise. If a laboratory conducts research that involves recombinant DNA, the principal investigator must register the research project with the university and the IBC assigns the project a biosafety level at which the work must be carried out. IBCs are authorized to conduct periodic safety audits to document compliance with the requirements for the project's laboratory biosafety level, biosafety work practices, and training requirements (DHHS 2009). These laboratory inspections entail a discussion of documentation of lab-specific training and standard operating procedures to ensure that records are up-to-date and reflect the types of experiments being carried out in the laboratory. For example, a typical university laboratory audit might note how microbes, chemicals, compressed gas, and hazardous waste are stored and handled; the state of the current equipment in the laboratory, and the laboratory itself, and whether the conditions impact safety; the presence of required emergency equipment (e.g., chemical spill kits, eyewash, safety shower); whether documentation on personnel training is up to date and if the laboratory possesses a chemical hygiene plan that includes a chemical inventory and standard operating procedures; the presence of relevant personal protective equipment; a risk plan that details experimental purpose, protocols used, types of infectious agents and route of infection, if necessary; annual biosafety cabinet inspections and certifications; a list of where all agents are stored; and whether appropriate signage is present in the laboratory(e.g., laboratory caution, emergency and waste guidelines).

If vertebrate animals are being used in the research, the project's principal investigator must develop a clearly articulated protocol to be filed with the Institutional Animal Care and Use Committee (IACUC). Protocols must be submitted to the IACUC for scientific and ethical review, and must be approved, prior to the initiation of any animal research. These protocols contain information regarding: experimental design (e.g., number of animals needed, how they will be treated, experiments to be performed and endpoints, pain category); personnel qualifications and training; justification for breeding, breeding methodology, and genotyping; emergency treatment and care (including euthanasia methods); and hazardous agents and how they will be used. In addition, annual updates on the approved protocol must be provided to the IACUC. These updates contain such information as the number of animals (living or dead), whether the protocol will remain active or will be terminated (and why), and if the research objectives have been met or changed. The National Research Council's Guidance for the Care and Use of Laboratory Animals, Eighth Edition, is an important science-based resource that scientists may draw upon as the develop pro-

[1]See the project website "Future Biotechnology Products and Opportunities to Enhance Capabilities of the Biotechnology Regulatory System": http://nas-sites.org/biotech/ [Accessed April 4, 2016].

tocols and carry out their research (NRC 2011). In addition, research must be conducted in accordance with the Animal Welfare Act, which regulates research on a number of live or dead "warm-blooded" animals, excluding birds, rats (*Rattus* species), mice (*Mus* Species), and food animals. As of May 2016, the committee is unaware of formal gene drive research proposals on animals that fall within the regulatory jurisdiction of the Animal Welfare Act.

Certain laboratory work on genetically modified plant species and "plant pests" is subject to federal regulations under the Biotechnology Regulatory Services of the Animal and Plant Health Inspection Agency (APHIS) of USDA. This body maintains jurisdiction over certain genetically modified organisms, particularly plant pests, including the transport of seeds or plants intended for laboratory use. The regulations are intended to help ensure that regulated genetically modified organisms are not harmful to plants or plant products by controlling the importation, interstate movement, or release into the environment of regulated organisms. Unauthorized (including accidental) importation, interstate movement, or release of a regulated article is a violation of the APHIS regulations (Plant Protection Act of 2000).

In sum, existing systems to govern biotechnology research in the laboratory include professional guidelines, institutional oversight committees that, in most cases, are accountable to federal agencies, and a process through which novel and controversial research can be considered by federal authorities before it proceeds. These systems are likely to have the flexibility to adapt well to gene drive technologies.

Governance Mechanisms for Phase 2 (Field Based Research) and Phase 3 (Staged Environmental Release)

Because US governance and regulatory considerations for Phase 2 and Phase 3 are similar, the following discussion applies to both phases, unless otherwise noted.

As noted above, regulatory authority for gene-drive technology will likely be dictated by the Coordinated Framework for the Regulation of Biotechnology. Ideally, the standards and regulations appropriate for field testing or environmental release of gene-drive technologies would be commensurate with potential harms, and take into account the extent to which a gene is expected to spread throughout the target population (e.g., Oye et al., 2014). However, as described below, the current U.S. regulatory system does not particularly account for the intentional spread of genetically modified organisms or their potential persistence in the environment. In addition, it is not clear how existing biotechnology regulations apply to gene-drive technologies.

Through its regulatory programs, APHIS has used its "plant pest authority" under the Plant Protection Act as the major tool for regulating biotechnology and releases into both contained and open areas. The Plant Protection Act also gives APHIS authority to regulate "noxious weed." APHIS is actively considering revising its rules to incorporate this additional authority into regulation, but to date has not done so (Pearson, 2015). Whether USDA can or will regulate a gene-drive technology such as the gene-drive modified Palmer amaranth in Case Study 6 (see Table 8-2) is unclear, because the noxious weed authority has not yet been translated into regulation.

For technologies that qualify, the APHIS-Biotechnology Regulatory Services system specifies permit conditions for field trials. These conditions are customized to the organism, trait, and release locations, in order to maximize confinement. Supplemental permit conditions can include a minimum separation distance to wild relatives and post-harvest monitoring requirements, among others. In 2014, USDA authorized close to 11,000 field trials of more than 12,000 types of genetically-modified organisms (Pearson, 2015). These organisms include insect plant pests, such as the pink bollworm and the diamondback moth, which have been engineered to suppress pest populations. APHIS draws a distinction between "containment procedures", which are used to prevent exposure of modified organisms to the environment, e.g., in laboratories, greenhouses, and during transport, and "confinement procedures" used during field trials to ensure the modified organism does not persist in the environment. The latter include reproductive isolation and post-harvest monitoring. For "contained" settings, the probability of release should be near zero; for "confined" settings, the probability of persistence in the environment should be near zero. Be-

cause some gene-drive technologies will be intended to persist in the environment, there is a clear mismatch with the current regulatory goal to prevent environmental persistence.

New engineering techniques are likely to lead to a higher number of genetically modified plants that will not be subject to USDA review (Carter et al., 2014). This is because APHIS's authority to regulate engineered plants relies on its "plant pest" authority. Even if APHIS were to add "noxious weed" authority to its biotechnology regulations, the limits are still likely to apply. This regulatory gap could mean that an increasing number of genetically modified plants may eventually be cultivated "for field trials and commercial production without prior regulatory review for possible environmental or safety concerns" (Carter et al., 2014). This result could also occur if the modifications are made using gene-drive technologies, although this is perhaps less likely because gene-drive applications are more likely to be aimed at the control of plant pests.

It is likely, but not certain, that FDA has the authority under the Federal Food Drug and Cosmetics Act (FFDCA) to regulate gene-drive modified organisms. The trigger for FDA oversight of gene-drive technologies would be the operable term "drug", defined in part as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals" or as "articles (other than food) intended to affect the structure or any function of the body of man or other animals." (Rudenko, 2015). The FDA's Center for Veterinary Medicine (CVM) is currently treats the genetic construct within an organism as a "new animal drug," requiring both premarket approval and post-approval oversight. The CVM states that "the [heritable] rDNA construct in a genetically engineered animal that is intended to affect the structure or function of the body of the genetically engineered animal, regardless of the intended use of products that may be produced by the genetically engineered animal, meets the FFDCA drug definition." In other words, it is the rDNA construct itself, and not the animal into which it has been inserted, that is considered a "drug" (FDA 2015a). Commercial entities wishing to market "regulated articles" under FDA's authorities over genetically modified animals must demonstrate that they are safe and effective.

However, the FDA has recently specified a definition of genetically engineered organisms that does not encompass modified insect disease vectors, modified invasive species, or many of the other types of applications likely to be relevant to gene drives. In its *Guidance for Industry 187: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs* (FDA 2015b), the FDA defines genetically engineered animals "as those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification." The guidance document also enumerates six classes of animals "based on the intended purpose of the genetic modification," as follows:

(1) to enhance production or food quality traits (e.g., pigs with less environmentally deleterious wastes, faster growing fish);
(2) to improve animal health (e.g., disease resistance);
(3) to produce products intended for human therapeutic use (e.g., pharmaceutical products or tissues for transplantation; these GE animals are sometimes referred to as "biopharm" animals);
(4) to enrich or enhance the animals' interactions with humans (e.g., hypo-allergenic pets);
(5) to develop animal models for human diseases (e.g., pigs as models for cardiovascular diseases); and
(6) to produce industrial or consumer products (e.g., fibers for multiple uses)."

The six criteria create some uncertainty as to whether the FDA has the regulatory authority to consider gene-drive modified organisms such as mosquitoes designed to prevent the spread of infectious disease in humans or animals (Case Studies 1, 2, and 3), or a mouse designed to reduce or eliminate nonindigenous mice on islands (Case Study 4). Despite the lack of clarity in the guidance, FDA is reviewing an Investigational New Animal Drug (INAD) application for a genetically engineered mosquito developed by the company Oxitec Limited more than ten years ago. The mosquito is designed to suppress wild populations of *Aedes aegypti*, a species that transmits a variety of human infectious diseases including dengue, chikungunya, Zika, and yellow fever. Since 2008, Oxitec pursued discussions with the USDA and

other regulatory agencies concerning the proper oversight of a field trial in Florida (Waltz 2015). Oxitec seeks to conduct a field trial in Key Haven, Florida. In March 2016, the FDA released for public comment the draft environmental assessment submitted by Oxitec (FDA 2016).

State and local laws, regulations and ordinances also contribute to the complex regulatory environment for outdoor research with gene-drive constructs in animals. Of greatest import may be the state-level environmental laws (e.g., the California Environmental Quality Act), and state and local notification requirements for the release of genetically modified organisms (e.g., Virginia Biotechnology Research Act Sec. 2.2-5500-5509).[2]

Gene-drive modified organisms released into the environment have the potential for trans-boundary movement. Governance will require communication and coordination between adjacent countries or states with separate regulatory jurisdiction. Both regional and national rules and regulations would apply. Laws and regulations at the country and local levels (nation, state, province, county, or lesser levels of jurisdiction control, such as a village) are also likely to play a significant role in the governance of the release of gene-drive modified organisms and their potential transboundary movement. The phase of staged environmental release, in particular, will have direct effects and implications for communities near and adjacent to the location of release, animating the issue of community participation in research governance.

Environmental Assessment and Public Consultation Under The National Environmental Policy Act

Like all other federal agencies, FDA and USDA/APHIS are subject to the National Environmental Policy Act (NEPA). NEPA requires agencies to determine if an environmental analysis is needed for a proposed action, and to assess impacts of those actions that have the potential to harm the environment (See Chapter 6 for additional discussion of the NEPA process). In the context of the Coordinated Framework, NEPA requires an *environmental assessment* (EA) to determine whether the introduction (field test of environmental release) of a specific biotechnology or related product has the potential to cause significant environmental effects, and inform federal government decisions whether to allow such an introduction. Federal agencies must prepare an Environmental Impact Statement (EIS) if a proposed major federal action is determined to significantly affect the quality of the human environment. The procedural requirements for an EIS are more detailed and rigorous than the requirements for an EA (40 CFR Part 1502).

Federal agencies can develop their own guidance for developing and evaluating environmental assessments. For example, APHIS performs EAs before providing permits for the release of modified organisms. The hazards of interest in such assessments include the potential for 1) a modified plant to become a weed in agricultural settings or to be invasive in natural habitats; 2) gene flow from the modified plant to sexually compatible plants whose hybrid offspring may become more weedy or more invasive; 3) the modified plant to become a plant pest; or 4) the modified plant to have an impact on non-target species. As is discussed in Chapter 6, EAs require supporting data to estimate impacts, but often the anticipated effects are not quantified as they would be in a risk assessment. Once a genetically modified organism is shown to lack hazardous traits and enters the commercial marketplace, it is no longer regulated by APHIS (Pearson, 2015).

Applications for products that are genetically modified animals are evaluated by FDA using what the agency calls a "risk based approach." FDA develops a specific set of questions about potential harms and benefits using a case-by-case approach for each product under evaluation. The intended application of the product drives the environmental assessment based on product definition, conditions of use, and other factors.

Two critical points need to be made in describing the potential role of NEPA and associated environmental assessments in the analysis of environmental effects of gene-drive modified organisms. First, to recap an important point from Chapter 6, while the preparation of an EA requires the assessment of potential impacts of the research activity, an EA does not require an ecological risk assessment. Thus, the

[2]http://law.lis.virginia.gov/vacodepopularnames/virginia-biotechnology-research-act/

necessary evidence to quantitatively estimate risk may not be gathered for environmental assessment procedures normally performed under NEPA.

Second, NEPA includes provisions for some public engagement. For environmental assessments, agencies sometimes take into account public views in the form of a public hearing or comment period. The INAD that Oxitec submitted for its genetically engineered mosquito includes an environmental assessment. The FDA issued a preliminary finding of no significant impact (FONSI) that agrees with the draft EA's conclusion. However, the FDA has said it will review public comments on the EA before issuing either a final EA and FONSI, or an EIS (FDA 2016). NEPA explicitly requires public consultation for an EIS. Through mandatory public hearings and comment periods, members of the public can express their views about the relative value of potential benefits and harms, and concerns about assumptions built into the environmental impact statement Thus, through provisions requiring transparent decision making and public input of various kinds, NEPA affords stakeholders and the general public the opportunity to participate directly in governance.

Some warn that the EA and EIS process "can be quite costly and time-consuming for the product developer" (Carter et al., 2014). NEPA has also been a tool for those who would use the courts to challenge an EA and FONSI and force a full EIS, which can delay matters for years and fundamentally alter the economics of a proposed innovation. Nevertheless, given the desirability of creating space for public engagement, NEPA would seem to be an important regulatory resource for the integration of public values into the governance processes.

Examining U.S. Regulation of Gene-Drive Modified Organisms through Case Studies

Which federal agency has the jurisdiction to approve field tests or environmental release of gene-drive modified organisms in the United States? Table 8-2 illustrates how the Coordinated Framework might apply to select case studies: Case Study 1 (gene-drive modified mosquito to combat dengue); Case Study 3 (using the house mosquito to combat avian malaria); Case Study 4 (controlling populations of nonindigenous house mice to protect biodiversity on islands); and Case Study 6 (controlling Palmer amaranth to increase agricultural productivity).

Notably, in all four cases, how gene-drive modified organisms fit within regulatory jurisdiction of FDA, USDA, and EPA is unclear, and their processes for assessing risks may differ from one another. In addition, there are many regulatory uncertainties, some of which have been listed in Table 8-2. For one example, if a gene-drive modified organism, such as the *Culex* mosquito (Case Study 3) has the potential to effect an endangered species such as honeycreeper birds (for good or for ill), what is the role of the Endangered Species Act and the U.S. Fish and Wildlife service, which has regulatory authority over actions that may affect the birds? A second example, is determining oversight for gene-drive modified organisms where there may be regulatory overlaps among the USDA, FDA, or EPA. In the case of a gene-drive modified mouse (Case Study 4), USDA could be considered the regulatory authority under the Animal Health Protection Act (7 U.S.C. §8301) if the mouse is considered a threat to animal health, or under the Plant Protection Act (7 U.S.C. §7701) if the mouse poses a threat to plants. The FDA could also be considered the regulatory authority for the mouse because the genetic construct (the T complex) used to develop a gene drive in the mouse might be considered an animal drug, because the T complex would be used to influence fertility. Although, it is clear that suppressing or eradicating a species population is not encompassed by FDA's six classes of animals "based on the intended purpose of the genetic modification." Finally, the EPA could be considered the appropriate regulatory authority under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. § 136 et seq.) if the wild-type mice are considered a pest, and the gene-drive modified mouse or the gene drive construct within the mouse is considered a "substance or mixture of substances intended for preventing, destroying, repelling, or mitigating" the wild-type pest (FIFRA § 2(u), 7 U.S.C. § 136(u)). A real world example of this confusion occurred with the genetically engineered mosquito developed by Oxitec. "The question of FDA versus USDA jurisdiction circled for years, until finally an understanding was reached: The FDA Center for Veterinary

TABLE 8-2 Potential US Regulatory Mechanisms to Oversee Environmental Release: Analysis of Selected Gene Drive Case Studies

	Case Study 1(mosquito)	Case Study 3 (mosquito)	Case Study 4 (mouse)	Case Study 6 (plant)
Application of the gene-drive modified organism	Reduce or eliminate the spread of dengue from mosquitoes to humans	Reduce the spread of avian malaria to threatened and endangered birds in the Hawaiian islands	Reduce or eliminate invasive mouse species from islands	Reduce or eliminate Palmer amaranth on agricultural fields in the southern United States
Regulatory authority under the current Coordinated Framework	FDA is likely to regulate genetic constructs within a gene-drive modified mosquitoes as "new animal drugs" as the agency has with the Oxitec genetically engineered mosquito; however it is unclear from the Coordinated Framework and guidance documents how that authority was determined.		Regulation of a gene-drive modified mouse could fall under any one of three agencies if mice are considered a plant pest (USDA), if the gene drive is considered a new animal drug (FDA), or if it is considered a pesticide/rodenticide (EPA)	The Plant Protection Act gives USDA the authority to regulate noxious weeds. The agency has not yet revised its rules to incorporate noxious weeds into their biotechnology regulatory authority
Agency-specific assessment under the current Coordinated Framework	Impact assessment under National Environmental Protection Act. If FDA assumes regulatory control, then they develop set of tailored assessment questions for each potential product		Without clarity of regulatory authority, assessment would be based on voluntary actions of research partnerships involved in development of the gene-drive modified mouse.	If USDA assumes regulatory control field tests and environmental release impact assessments would be conducted under that National Environmental Protection Act
Select Regulatory Uncertainties	**Differences in agency approach to assessing harms, public consultation, and other components of decision-making** In Case Study 4, for example, regulation of a gene-drive modified mouse as a rodenticide under the EPA, would likely trigger agency policies for ecological risk assessment, a quantitative and much more rigorous assessment than environmental impact assessments that FDA and USDA might carry out under the auspices of NEPA. **The role of agencies with authority over sites where gene-drive modified organisms might be released or over species that may be affected by the release.** In Case Study 3, for example, what is the role of US Fish and Wildlife, which has authority over endangered and threatened honeycreeper birds and USDA Forest Service, which has authority over much of the Hawaiian forests where endangered birds reside? **The role of tribal governments in the decision making process for the field testing or release of gene-drive modified organisms on or near tribal lands**. It is uncertain how institutional decisions regarding gene drives will be integrated with tribal governance frameworks to ensure justice and respect. In Case Study 1, for example, if dengue moves into the southern United States, who has authority to determine whether gene-drive modified mosquitoes could be released in or near tribal lands? **Mechanisms in place for international considerations and coordination for field testing or release of gene-drive modified organisms near national borders.** In Case Study 6, for example, Palmer amaranth is a weed in the southern United States that can interbreed with related plant species that are cultivated as vegetable crops in Mexico. What mechanisms are in place for dialogue with the Mexican national government? How will any concerns raised by the Mexican government be incorporated into U.S. decision-making processes?			

Medicine would be the lead agency coordinating other federal and state agencies, [but] by then, Oxitec had begun trials in South America and the Caribbean." This Oxitec case demonstrates both a major challenge (years of delay) when there are overlaps in regulatory jurisdiction and a potential solution, creating a process to quickly develop memorandums of understanding between federal agencies when regulatory jurisdiction is uncertain. As a third example, the mechanisms to solicit input from communities that live in or near potential sites for field testing or environmental release of a gene-drive modified organism are unknown. NEPA mandates public involvement to include, at a minimum, "reasonable public notice" of environmental assessments," but it is not clear which mechanisms each agency would use in the case of a gene-drive modified organism or how public input would be incorporated in the decision making process is not clear. In addition "reasonable public notice" falls short of the engagement that is needed for gene-drive modified organisms (see Chapter 7).

BIOSECURITY CONSIDERATIONS

An area that will need continual discussion and evaluation is the biosecurity and related uncertainties of gene drive research. It is assumed that efforts to introduce a gene drive into an organism are performed with good intent, that ethical and regulatory standards will be followed, and that the necessary review and approval by oversight committees will be sought. However, concerns related to gene drive technology include not only unintended or unanticipated effects, but also the potential for the unethical, intentional creation of an organism with the capacity to spread undesirable traits into a population. As an illustrative case study, in late 2011, manuscripts by two independent research groups describing research on the highly-pathogenic avian influenza H5N1 that increased the transmissibility of the virus (Imai et al., 2012; Herfst et al., 2012; Russel et al., 2012) gained the attention of the United States National Science Advisory Board for Biosecurity; the concern that the studies could turn H5N1 into a bioweapon resulted in a worldwide moratorium on the research and legal battles to get manuscripts published.

A United States government policy for oversight of dual use research of concern in the life sciences developed in 2012 was modified in 2014 to include new requirements for oversight and training (S3 2014). Research involving any of 15 agents or toxins must be reviewed in the context of dual use potential, and it is possible that gene drive research could fall under one of the seven categories of experiments listed in the policy. As described in Chapter 5, planning research that involves genetically engineering mosquitoes requires multiple steps with associated guidelines (including for physical and biological containment), regulations, and laws that determine progress from concept to release. However, engineering that includes the introduction of a gene drive may require modification of current governance and perhaps the implementation of review criteria that to date have not yet been applicable to the field. Although they seem unlikely, examples of possible scenarios where dual use might apply are described below.

Gene drives are likely to raise similar biosecurity concerns as those raised in the discussion of genetic modification and synthetic biology techniques. In these cases, state-sponsored terrorism is considered to be the most serious threat to biosecurity and also to be the most difficult to pre-empt. Reports dealing with the governance of synthetic biology have cited a range of precautionary measures to address biosecurity threats (Lowrie and Tait, 2010; Presidential Commission for the Study of Bioethical Issues, 2010; IRGC, 2011). These reports have also pointed out that the most effective means to deal with such threats should they materialize is to use the relevant scientific expertise to develop rapid diagnostic techniques and synthesis methods for vaccines and antibiotics to enable a fast response to a threat (IRGC, 2011, Presidential Commissions, 2010). The availability of rapid diagnostic and synthesis technologies will also enable states to respond rapidly to the much more likely threat of a naturally-occurring emerging disease or a future pandemic.

There are several types of concerns related to safe, ethical, and secure research:

- Unintended and unforeseen consequences of release;
- Unintended releases due to negligence or natural disasters;

- Release of information that could be used for intentional misuse; and
- Intentional release or misuse of a gene-drive modified organism.

Unintended consequences or releases are the domain of *biosafety*. In general, scientific norms and institutional guidelines on biosafety adequately address these issues. The potential for misuse of research, however, is the domain of *biosecurity*. As noted by the International Academy Panel (IAP), it is difficult to predict the outcome and consequences of research; nonetheless the potential for misuse must be "anticipated and minimized to the extent possible in the planning, performance, and dissemination of research" (IAP, 2016). The IAP emphasizes that scientists have a responsibility "to participate in discussions about the possible consequences of their work, including harmful consequences, in planning research projects."

Intentional Misuse

Gene drive research has advanced considerably for mosquitoes (see Chapter 2). The impetus for genetically engineering mosquitoes is to control mosquito-borne diseases, either by suppressing mosquito populations or by replacing existing wild populations with mosquitoes that have a reduced capacity to be infected with or transmit a pathogen, such as dengue viruses or *Plasmodium* species that cause malaria. As described in preceding chapters, a number of excellent guides are available to ensure that researchers working to genetically engineer mosquitoes follow ethical steps from concept to application and are performing these experiments in situations and facilities that protect the public and minimize the risk of accidental release into the environment. Although the committee firmly believes that members of legitimate research community working on gene drives do so ethically and work with the intent to benefit society, for the sake of completeness, the possibility that there may be researchers (or regimes who control research agendas) whose motivation is to cause harm needs to be considered. Given the current understanding of the genetics of vector competence, using gene drives in mosquitoes for malicious intent would seem to be extremely difficult from a technical standpoint, making gene drive research an unattractive proposition compared with other options for causing harm. Yet, with a better understanding of the basis of mosquito-pathogen interactions, it is not inconceivable that rather than developing a resistant mosquito, one could develop a more susceptible mosquito capable of transmitting a specific pathogen more efficiently than wild-type mosquitoes. It might even be possible to develop mosquitoes that could transmit a pathogen that is not normally vector-borne, or that could even be able to deliver a toxin. The latter might be accomplished by engineering a gene encoding a toxin with a secretion signal under the control of a salivary gland gene. Unlikely as this may sound, early discussions on applications for genetically engineered mosquitoes included expression of heterologous proteins to vaccinate the humans on whom they fed (Crampton et al., 1999) and a patent was issued to protect this technology.[3] As a proof of principle, Kamrud and colleagues (1997) infected mosquitoes with a viral expression vector and were able to detect a marker in the mosquito saliva. Other researchers used a similar approach to express a toxin gene in mosquitoes, although the location of the protein in specific tissues was not attempted because toxin expression at very low levels rapidly killed the mosquitoes (Higgs et al., 1995).

The actual and potential use of insects as weapons has been discussed; for example, by releasing insects infected with human pathogens or releasing agricultural pests (Lockwood, 2012). However, the availability of a gene drive provides a new opportunity for malicious use because its self-sustaining nature poses a perhaps more significant threat. In the context of such research being performed in an academic setting, such experiments would be subject to scrutiny via the IBC review process. Since September 2015, if the reviewed research meets certain criteria, the research institution is required by the Department of Health and Human Services (HHS) government policy (S3 2015) to determine whether the proposed research should be designated as Dual Use Research of Concern.

[3]Delivery system US 20030192067 A1, Inventors Robert Sinden and Julian Crampton. For more information, see http://www.google.com/patents/US20030192067.

Research to introduce a gene drive into mosquitoes could conceivably be interpreted as meeting experimental criteria included in the HHS dual use policy. Such criteria apply to research that, for example, could disrupt immunity or effectiveness of immunization, could increase the transmissibility or ability to disseminate an agent or toxin, or could alter the host range or tropism of an agent or toxin. Mosquito salivary gland proteins can influence immune responses of the vertebrates on which they feed and can influence pathogen establishment and diseases development (Schneider and Higgs, 2008). Moreover, since it may be possible to engineer mosquitoes to be more efficient vectors, which in effect increases the transmissibility of pathogens, it is probable that some approaches to genetically modifying mosquitoes may constitute dual use research of concern. As stated above, this discussion applies in the context of developing a gene-drive modified mosquito with good intent; however, just as there are inadequacies associated with the Cartagena Protocol with regards to oversight and jurisdiction, those who would deliberately create modified mosquitoes with malicious intent will likely operate outside of the purview of ethics, biosafety, and other review committees. In the 2016 Worldwide Threat Assessment of the US Intelligence Community, the US Director of National Intelligence classified genome editing as a weapon of mass destruction and proliferation (Clapper, 2016).The assessment states "given the broad distribution, low cost, and accelerated pace of development of this dual-use [genome editing] technology, its deliberate or unintentional misuse might lead to far-reaching economic and national security implications" (p. 9). The impact the Worldwide Threat Assessment may have on gene drive research is not yet known.

There are well-developed resources and guidelines adequate to enable safe and secure research with appropriate oversight in, for example, academic environments in which research is being performed. Manipulation of mosquitoes should be performed in arthropod containment level two (ACL-2) insectaries at a minimum, which fulfill facility design criteria, with appropriate standard operating procedures and adequately trained personnel. In addition, the NRC publication *Understanding Biosecurity: Protecting Against the Misuse of Science in Today's World* details the role of the scientific community and governments in preventing misuse (NRC, 2010. Box 8-4 summarizes key concepts in the report (NRC, 2010) that are relevant for gene drives.

GOVERNANCE OF GENE DRIVES IN GLOBAL CONTEXTS

International sources of governance that may apply to gene drive research have as much impact on whether and how science develops, as do national the United States' sources of domestic governance. As gene drive research advances, the scientific community and regulators will need to consider mechanisms and policies for global engagement for two main reasons. First, gene drive science is a global endeavor. The early stages of gene drive research (e.g.., phase 1 and phase 2, See Figure 5-1) are predominantly taking places in high income countries like the United States, the United Kingdom, and Australia. However, later phases of research—contained and open field tests—are likely to take places in other parts of the world. One example is the use of gene-drive modified mosquitoes to combat human malaria (Case Study 2), a disease that disproportionately impacts the tropics and the southern hemisphere, particularly in low- and middle-income countries. Field trials are most likely to take place in countries where malaria is endemic just as related research on the use of *Wolbachia* and the RIDL mosquito to combat dengue has been concentrated in these countries. However, some of the jurisdictions targeted for field testing or releases may lack the capacity to assess safety of experiments in a scientifically and socially robust fashion.

Second, the unique qualities of gene-drive modified organisms to spread and persist in the environment will require any nation planning field tests or environmental releases to consider whether and how gene-drive modified organisms will cross national borders. As noted previously, for example, Palmer amaranth is a damaging weed in the United States (Case Study 5), but a related *Amaranthus* species, with which Palmer amaranth can interbreed, is cultivated for food in in Mexico, South America, India, China and Africa. The escape of a suppression drive in Palmer amaranth could affect non-targeted species and negatively impact valued *Amaranthus* vegetable crops. There are currently no national regulatory mechanisms worldwide that adequately address field testing and environmental releases of gene-drive modified

Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, & Aligning Research with Public Values

BOX 8-4 Key Concepts to Protect Against Misuse of Scientific Research

Understanding Biosecurity: Protecting Against the Misuse of Science in Today's World (NRC, 2010) emphasizes that scientific progress combined with globalized nature of research and societal interactions, has expanded vulnerability to misuse and outlines roles and responsibilities for members of the scientific community to prevent misuse. The report also points out that the opportunity to advance research for legitimate purposes is paired with the responsibility to reduce the potential for some materials, knowledge, tools, and technologies to be used to do harm. Scientists, research institutions, journal editors, professional societies, and governments all play important roles and responsibilities to encourage research and mitigate the potential for research to be misused.

First, the report describes how scientists are the "front-line defense" against the misuse of research. To bolster this front-line defense, scientists should be cognizant of the societal implications of their work, including potential applications *and* potential misuses, and actively educate policy makers who focus on security research about those implications. The scientific community should also continue to develop and improve upon existing guidelines that encourage new lines of research and deter potential misuses. One approach is a cradle-to-grave system (i.e. phase 0 through phase 4 in the phased testing scheme presented in Chapter 5), in which security issues are identified when research is first proposed and in every subsequent stage through the publication of research results.

Second, the report recommends that research institutions protect the scientists working in their facilities as well as the communities in which the research facilities are located. Important mechanisms to ensure biosafety and biosecurity are Institutional Biosafety Committees, Institutional Review Boards, and Institutional Animal Care and Use Committees. Research institutions should also facilitate exchanges among scientists and others, for example by working with federal agencies to develop opportunities for scientists to participate in policy fellowships at national intelligence and security agencies and for members of the intelligence community to participate in fellowships at universities.

The U.S. federal government oversees potential misuse of science and technology through two primary mechanisms: the Select Agent Program and the National Science Advisory Board for Biosecurity.

To strengthen international efforts to prevent the misuse of science, the report emphasizes the need for both bottom-up, scientist-driven guidelines and practices, and top-down standards and policies from research institutions and governments. The report encourages international scientific organizations such as the International Council for Science, InterAcademy Panel of International Issues, Academy of Sciences for the Developing World to play a role in bottom-up solutions.

organisms. Scholars of governance warn that the regulation of new technologies with societal implications will require evidence-based policy processes, with deliberate and participatory engagement in policy making by the people who will be impacted by these innovations (Lyall and Tait, 2005).

Gene drive research will require international collaborations, and attention should be given to the research capacity and biosafety regulations in other parts of the world, particularly in low- and middle-income countries. Reconciling differences between preventative and permissive regulatory schemes, as described earlier in this chapter, will likely be a considerable challenge for the international development and testing of gene-drive modified organisms. The difficulties introduced by widely divergent regulatory systems are compounded by the potential for these organisms to spread across state and national borders. Careful consideration will need to be given to whether national differences in approaches to governance will create gaps in the ability to protect human health and the environment, or whether such differences could impede basic research that does not yet have clear benefit for society. For these reasons, responsible systems of governance will need to incorporate clear mechanisms for international dialogue among governing authorities, and perhaps, formal or informal agreements about the use of potential gene-drive technologies and comparable standards for biosafety.

International cooperation and attempts to harmonize research standards for science and technology is not a new endeavor. Policy tools that span guidelines for research to legally enforceable treaties have been considered and developed for many areas of science, such as stem cells research, climate change,

and nanotechnology. In general, there are three commonly used governance mechanisms for international agreements: policy, international coordination and cooperation, and formal treaties (Breggin et al., 2009; see Table 7-3). In the 2015 International Summit on Human Gene Editing (National Academies of Sciences, Engineering, and Medicine, 2016), Gary Marchant, Professor of Emerging Technologies, Law and Ethics at Arizona State University, laid out a number of disadvantages and advantages to international systems of governance (Marchant, 2015). Marchant observed that it is difficult to integrate social, political, and ethical norms of different countries into a single policy, and that developing international systems of governance may require substantial resources that may take away from developing strong national-level oversight. On the other hand, the benefits of internationalization include standards that provide consistent requirements for scientists and their research institutions, and such standards could ensure equal protection for citizens of all nations. Marchant noted that it is difficult to develop international harmonization of governance when some countries lack national regulations; nonetheless developing national regulations in every country before putting harmonization mechanisms in place may unduly delay international agreements and be more difficult in the face of entrenched and inconsistent national regulations, such as those on genetically modified organisms.

Two relevant sources of international governance of gene drive research are the United Nations Convention on Biological Diversity (CBD) and the WHO's Guidance Framework for Testing Genetically Modified Mosquitoes. Neither CBD nor the WHO explicitly addresses gene drive research, although discussions are underway.

The Convention on Biological Diversity and its Protocols

The United Nations Convention on Biological Diversity is the main international regulatory instrument governing the development and use of genetically modified organisms. The CBD is a multilateral treaty focused on the global conservation of biological diversity. To date, 193 states are parties to the Convention. The objectives of the Convention are threefold:

- Conservation of biological diversity;
- Sustainable use of the components of biological diversity; and
- Fair and equitable sharing of the benefits arising out of the utilization of genetic resources.

Parties to the Convention[4] are required to "establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health" (UN 1992).

TABLE 8-3[a] Types of International Agreements

Policy Tool	Definition	Example
Policy	Informal communications and policy learning between regulators	US – UK Agreement for Scientific and Technological Cooperation
International coordination and cooperation	Formal or informal congruent approaches without large-scale adjustment of domestic law and regulation	World Health Organization Guidance Framework for Testing Genetically Modified Mosquitoes
Treaties	Formal negotiated agreements on common rules and standards for domestic regulation	Convention on Biological Diversity

[a]Based on Breggin et al. 2009.

[4]To become a Party to the CBD and its protocols, a nation must first have gone through a process of ratification, acceptance, and approval or accession, after which it can take part in decision making processes, and is also obliged to pass national laws implementing CBD provisions.

The Convention on Biological Diversity is implemented through its two protocols (international agreements), the *Cartagena Protocol on Biosafety* and the *Nagoya Protocol (NP) on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization* . Although the Convention itself does not strictly police compliance, many of the Parties have regulatory systems, developed under the Convention and its protocols, that are based on a strong precautionary, near preventative approach, and implement its provisions in a way that is seen by some to be overly restrictive of these technologies, the EU being the most prominent example (Strauss et al. 2009; Freeman and Swidick 2013).

The Cartagena Protocol was developed primarily because of concerns related to genetically modified crops, with the purpose of addressing potential risks posed by releases of genetically modified organisms into the environment (CBD 2016). However, potential extensions of the powers of the Convention in the governance of gene drive research, and, relatedly, synthetic biology, are being explored. A 2012 report of an ad hoc technical group on risk assessment includes discussion on mosquitoes modified with a gene drive (CBD 2010). In addition, the 2012 "Guidance Document on Risk Assessment of Living Modified Mosquitoes (CBD 2012)," recognizes that "In cases where living modified mosquitoes are modified with gene drives, containment may not be possible even when efforts are made to reduce long-distance dispersal due to anthropogenic activities". In 2015, the Open-ended Online Forum on Synthetic biology was held to inform work of Ad Hoc Technical Expert Group. For the Forum, the Conference of the Parties (COP) invited Parties, other governments, relevant international organizations, indigenous and local communities, and other relevant stakeholders to submit information on seven topics related to synthetic biology to the Executive Secretary. The Forum demonstrated that there is a broad range of differing opinions, between and within nations, about the operation of the Convention, and about how its current provisions would relate to the governance of synthetic biology (CBD, 2015), and by extension to gene drives.

At the time of the adoption of the Convention on Biological Diversity and the Cartagena Protocol, there were good reasons to take a cautious approach to the potential harms to biodiversity that might arise from the development of genetic engineering technologies, particularly in agriculture. However, there is a growing body of evidence that genetically engineered crops deliver many significant benefits for agriculture, particularly for resource-poor farmers, as well as for biodiversity and ecosystem services (ISAAA, 2015). The apprehension about the extension of the powers of the Convention and its protocols to cover synthetic biology, expressed in the consultations referenced above, relates to the lack of adaptation of the strong precautionary approach in light of the evidence we now have for safety and benefits. Indeed, within these consultations, there are continuing calls for additional enhanced levels of restraint for use of genetically engineered crops themselves and particularly for synthetic biology, including calls for a moratorium on all forms of synthetic biology research, even in contained use.

The challenges for gene drive research arising from the Convention and the Protocols lie mainly in the way in which individual countries choose to implement their provisions, rather than in the provisions themselves. Concerns about the impacts of these regulatory provisions on future innovative developments are based on assumptions that there will be no future downwards adaptation of regulatory provisions based on experience in use of new technologies. Indeed, these discussions and consultations are being seen by some as an opportunity to reinforce and extend a preventative emphasis.

Many low- and middle-income countries are signatories to the Cartagena Protocol, which has guided the development of their national regulatory frameworks for governance of living modified organisms, which it defines as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Under the Cartagena Protocol, countries are obligated to notify one of the U.N.'s International Biosafety Clearing-Houses and any affected nations about activities that may lead to movement of living modified organisms with potential adverse effects on biological diversity or human health. However, some countries do not have sufficient resources to enforce such legislation. As a result, capacity building and public awareness activities in low-income countries have largely been top down, with governments playing a passive role and non-governmental organizations taking up the brokering role (Kingiri and Hall, 2012).

Some countries have developed regional regulation in order to assist individual countries with adoption of the Cartagena Protocol. For example, The African Model Law on Biosafety (2011) aims to help

countries that are members of the Cartagena Protocol on Biosafety to implement the provisions of the Protocol at the national level (Figure 8-2). Paarlberg (2012) argues that African countries in particular have largely followed the European Union's precautionary approach, which limits the deployment of biotechnology. Furthermore, politics can delay deployment of promising innovations as has occurred in the Philippines (Brooks, 2010; Kupferschmidt, 2013), Kenya (Paarlberg, 2001; Paarlberg, 2009; Brooks, 2010; Zhu, 2014) and India (Herring, 2008; Jayaraman, 2010) where field trials of genetically engineered crops have been destroyed or disrupted.

These experiences point toward the need for governance mechanisms that recognize the urgent need to improve poor countries' ability to exploit new scientific and technological advances to address social and economic challenges affecting the poor (Watkins and Ehst, 2008; Juma, 2011).

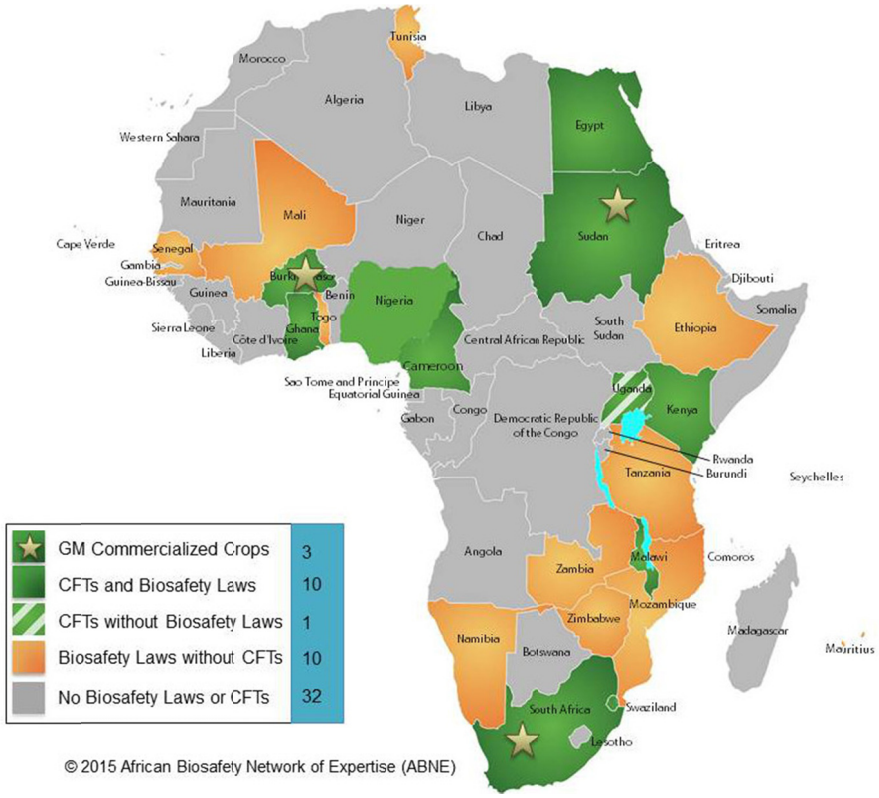

FIGURE 8-2 The Status of Biosafety Laws on Genetic Engineering in African Countries. CFT = confined field trials; GM – genetically modified. Source: African Biosafety Network of Expertise. The African Model Law on Biosafety (2011) has provisions for establishment of institutional arrangements for the development of national focal points, competent authorities, national biosafety committees, and IBCs. Thirty-seven countries in Africa are signatories of the Cartagena Protocol. Notably, the United States, Canada, Côte d'Ivoire, Sierra Leone, Australia, Argentina, and Chile, among a few other countries, are not parties to the Cartagena Protocol, and so they are not subject to its provisions. However, these countries are impacted by the ability of those that are parties by requiring compliance with their national regulations adopted under the requirements of the Cartagena Protocol, and by other international instruments and trade agreements. In other words, the United States is not a party to the CBD and its protocols, but their wide adoption of the CBD internationally will have an impact on trade-related opportunities and the ability of United States based researchers to conduct field testing or environmental releases in other countries. This may raise the prospect of disputes and the need to consider legal authorities in multiple jurisdictions as well as international law. "It is also expected that genetically modified mosquito trials will require formal authorization by relevant government authorities in recognition of the country's sovereignty" (WHO, 2014, p. 79). We are at a point where gene drives already necessitate that discussion, which needs to be translated into clear statements of international expectations even if no mechanism exists to enforce regulations universally.

Transnational Governance through Standards and Guidelines

There is currently no overall framework that policy makers and standards bodies can use to assess what mix of standards, guidelines, and regulations is appropriate for transnational governance of any particular technology. In his presentation at the 2015 International Summit on Human Gene Editing, Gary Marchant discussed the idea of "transnational new governance." Transnational new governance originates from a "soft law" concept in international law. It entails substantive obligations and requirements created by instruments that are *not* directly legally enforceable. These instruments have an international scope, focus, and participation and can broaden oversight from top-down government requirements to include a much broader range of decision makers, for example, companies, researchers, NGOs, public-private partnerships, and other third parties. Their advantages include the fact that they are voluntary, cooperative and reflexive; can be adopted or revised relatively quickly; allow many different approaches to be tried simultaneously: and can be gradually "hardened" into more formal regulatory oversight (Allen and Sriram, 2000; Langlois and Savage, 2001). They do, however, have limitations. For example, their norms and standards are not directly enforceable; they are not always as flexible and adaptable as hoped; there is potential for confusion and overlap; and they have less legitimacy. Examples of transnational non regulatory and non-legislative governing tools are provided in Table 8-4. In transnational new governance, a number of respected, non-regulatory authorities, such as the International Council for Science, InterAcademy Partnership[5], Academy of Sciences for the Developing World, and the WHO, may have important roles to play in shaping responsible practices for gene drive research internationally.

TABLE 8-4 Examples of Transnational Governance Tools in Science that are Non Regulatory and Non-Legislative

Policy Tool	Example
Transnational regulatory dialogue and networks	Working groups of the Organization for Economic Co-operation and Development
International harmonization committees	International Conference on Harmonization
United Nations declarations	International Declaration on Human Genetic Data
International principles	World Medical Association, Helsinki Principles
International scientific assessment	Intergovernmental Panel on Climate Change
Research guidelines developed by international professional scientific societies or other non-regulatory science authorities	International Society for Stem Cell Research Guidelines for Embryonic Stem Cell Research World Health Organization's Guidance Framework For Testing Genetically Modified Mosquitoes World Organisation for Animal Health (OIE) – Ensuring Good Governance to Address Emerging and Re-emerging Animal Disease Threats Food and Agriculture Organisation (FAO) – Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues International Committee of Medical Journal Editors
International statements of policy	Human Genome Organization's statement on the patenting of DNA sequences
Private/industry standards	International Standards Organisation International Gene Synthesis Consortium's Harmonized Screening Protocol

Source: Adapted from Marchant, 2015.

[5]InterAcademy Partnership (IAP) is a global network of the world's science academies, launched in 1993: http://www.interacademies.net/.

There is also an increasingly well recognized capacity for guidelines and standards to supplement and contribute to the implementation of regulations in order to support the speedy and effective delivery of science that safely meets the public's needs and desires; and, where appropriate, contribute to the development of a supportive innovation environment (SBLC, 2016). In other words, such guidelines and standards can have a *formative role* in the very early development of radically innovative technologies to guide future regulatory decisions (Figure 8-3). Thus, an innovation such as a gene drive, in early stages of development (Phase 1), could most appropriately be governed through the adoption of a of consensus standard designed to set an agreed level of good practice or quality or establish trust in an innovative product or service. This agreement would be a preliminary step to better informed development of more formal regulatory guidelines (Phase 2 in Figure 8-3) and then, as understanding of the new technology and its properties increases, to legally-based regulations (Phase 3). At Phase 4, once the properties of a novel technology have been fully codified and understood, standards will continue to serve their traditional *subsidiary role* to regulations, for example, to ensure quality and safety of products and good manufacturing practice (Table 8-4).

Although not a regulatory body, the World Health Organization (WHO) serves as the *de facto* authority to its member states (194 countries worldwide), regarding which interventions have demonstrated public health value and therefore should be considered for adoption by Ministries of Health. The WHO has been instrumental in facilitating the generation of normative guidance and standards for research, biosafety, and risk assessment on vector control paradigms. A series of guidelines with outlined testing protocols and pre-defined efficacy standards are available at no cost to investigators, industry, and other stakeholders, their purpose being to ensure that proven, effective products are used in the field. Such resources are particularly valuable to low- and middle-income countries where regulatory infrastructure may be lacking but in-country decision makers/authorities recognize the need to comply with the Cartagena Protocol. Many countries, particularly those who lack national or regional governance mechanisms, look to these guidelines to develop their biosafety governance systems. The WHO also manages expert panels to encourage self-governance. For example, the Vector Control Advisory Group (VCAG) was established in 2012 to provide expert advice to investigators on the evaluation and development of vector control products and strategies. The VCAG serves as a venue to vet scientific concepts, appropriate study designs, efficacy needs, and considerations on decisions points for advancing evaluation from one phase of testing to the next. The expert advice facilitates the generation of rigorous findings and provides a foundation for science-based decisions points. All of VCAG's critiques and comments are posted in the public domain for full transparency to enhance VCAG's engagement with a broad range of stakeholders that may be impacted by the research and/or implementation of specific strategies.

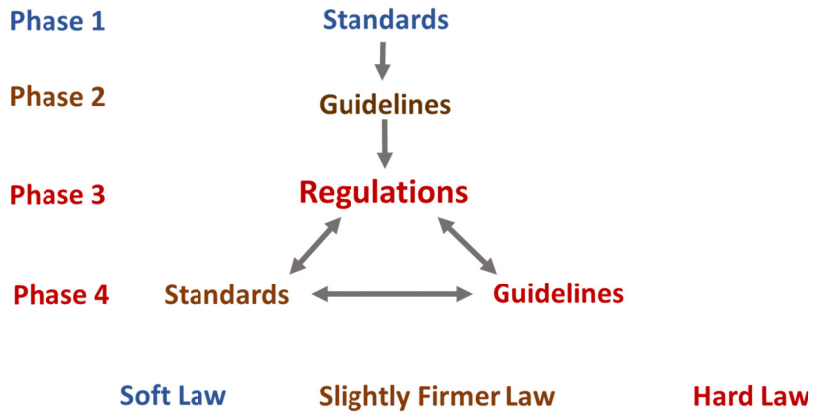

FIGURE 8-3 Roles of Standards and Guidelines in Interaction with Regulations. SOURCE: Adapted from Tait and Banda (in press), Normative Guidance from the World Health Organization.

Most relevant to this report, the WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR) has coordinated specific efforts to develop internationally accepted guidelines for the testing of Genetically Modified Mosquitoes (GMM). *The Guidance Framework for Testing of Genetically Modified Mosquitoes* (WHO, 2014[6]) highlights the need for a staged-approach to the evaluation of GMM to ensure evidence-based decision points are utilized for further development of the strategy. A complementary training manual, *Biosafety for Human Health and the Environment in the Context of the Potential use of Genetically Modified Mosquitoes (GMMs)* (WHO/, 2015[11]), provides investigators of GMMs a tool for governing their research as it relates to mitigating risk of accidental filed-based trials or open environmental release.

Many countries, particularly those that lack national or regional governance mechanisms, look to the WHO's GMM arthropod containment guidelines to develop their biosafety governance systems for mosquitoes. It is expected that many of the WHO's normative principles for evaluating GMMs will also apply to gene-drive modified mosquitoes; however, the challenge with emerging gene-drive technology, is that not all aspects of the WHO GMM principles may apply. This may lead to challenges for considering gene-drive modified mosquitoes and other gene drive modified organisms.

The WHO's Special Programme in Research and Training in Tropical Diseases (WHO/TDR) also funded a project to produce *Best Practice Guidance for Deployment of Genetic Control Methods against Mosquito Vectors in Disease Endemic Countries* (MosqGuide) (Mumford et al., 2009). This initiative is already engaging Panama, Brazil, Mexico, Thailand, Kenya, and India.

In some of the jurisdictions targeted for experimentation, there may be no governance system in place or only one that lacks the capacity to assess experiments in a scientifically and socially robust fashion. International organizations like the WHO are attempting to address this problem by promoting ethical codes and best practices that might be used in these situations.

A number of challenging questions for governance in global contexts remain. Is local review by authorized committees in host countries required for an experiment to proceed ethically? Is prior consent at the national level necessary? International standards raise the potential problem of legitimacy gaps. If international organizations are filling the governance gap at the international level, what procedures are being used to produce those standards, and who should participate in that process?

CONCLUSIONS AND RECOMMENDATIONS

The governance of research begins with the personal responsibility of the investigator, is formalized in professional guidelines, and often extends to legally binding policies and enforceable regulations. In the United States, it is clear that gene drive activities will trigger a variety of governance mechanisms. However, some of these mechanisms may be inadequate for identifying immediate and long-term potential environmental and public health implications of individual gene-drive applications because they lack clarity in their jurisdiction, they are challenged by the novel characteristics of gene drives, or they provide insufficient structures for public engagement.

Currently, institutions, funders, and professional societies work in concert to encourage professional best practices in research, and this cooperation will be key to maintaining high standards. Professional codes of conduct that address technical and ethical considerations in research are an important source of governance that helps both to promote awareness among researchers and encourage them to take responsibility for their science. Approaches currently in use to incentivize and refine good practices are to provide resources for education (conceptual) and training (practical) in the responsible conduct of research and to publically acknowledge researchers for their standards of practice. These will be important for reinforcing responsible practices in gene drive research.

Laboratory-based research conducted at an institution that receives funding from the NIH is subject to NIH's guidelines on biosafety and oversight by IBCs. These guidelines, although international in nature, are adapted to specific institutional contexts and are complemented by good laboratory practices.

[6]WHO/TDR annual reports can be found at http://www.who.int/tdr/publications/about-tdr/annual-reports/en/

Moreover, the NIH guidelines stipulate that all research at NIH-funded institutions may be regulated by laws established at the local, state, and federal levels, even in the absence of NIH funding for a specific project (e.g., other federal agencies, private foundations).

Over the last few decades, IBCs have provided a robust system of health and environmental protection for laboratory research. Part of the advantage of an IBC is its flexibility: reflected in the use of guidelines that can be modulated as technology and experience develop, a delegated system of oversight that operates at the local level but is accountable to a governmental body, and a process through which novel and controversial research can be considered at a level higher than the research laboratory.

Although these features of IBCs will be useful as gene drive research moves forward, IBCs also have important limitations. Due to the novel characteristics of gene drives, capacity issues, and an absence of clearly defined guidelines for gene drive research, current IBCs may not have the expertise or resources to evaluate the biosafety of gene drives effectively. IBCs are also not equipped to examine biosecurity or willful misuse issues. However, there is potential to learn from IBCs at institutions where gene drive research has been ongoing.

At the institutional level, it is essential that gene drive research continue to be governed by good professional practices, strict adherence to standard operating procedures, and comprehensive training of research personnel.

Recommendation 8-1: Institutions, funders, and professional societies should provide face-to-face instruction and online, open access resources for education and training on the responsible practices in gene drive research.

Recommendation 8-2: Due to the novel characteristics of gene drives, funding agencies and research institutions should take responsibility to ensure the development of the necessary expertise to assess safety within Institutional Biosafety Committees and their equivalents.

Each phase of research activity—from developing a research plan to post-release surveillance—raises different levels of concern depending on the organism being modified and the type of gene drive being developed. A one-size-fits-all approach to governance is not likely to be appropriate. Governance and regulation of gene drive research will need to be proportionate to the hazards posed by the specific activity. In addition, governance will need to be responsive to changes in scientific best practices and ethical considerations as gene-drive technologies develop.

Recommendation 8-3: Researchers and funders should take measures to review the study design and implementation on an ongoing basis to ensure that risks and benefits remain reasonably distributed and balanced.

In the United States, regulation of gene-drive modified organisms will most likely fall under the Coordinated Framework for the Regulation of Biotechnolgy. However, the US Food and Drug Administration (FDA), the US Department of Agriculture (USDA), and the US Environmental Protection Agency (EPA), the federal agencies included in the current Coordinated Framework, do not have clear lines of authority over the potential applications of gene drive research. The diversity of potential gene-drive modified organisms and contexts in which they might be used reveal a number of regulatory overlaps and gaps. For example, regulatory practices for the assessment of potential ecological and public health effects of field experiments or planned releases are inadequate for gene drive research due to these policies' predication on containment. For some potential applications of gene-drive technologies, regulatory jurisdiction may overlap, which suggests the need for a process to quickly determine which agency should coordinate governance of that technology.

Recommendation 8-4: The U.S. government should clarify the assignment of regulatory responsibilities for field releases of gene-drive modified organisms, including the roles of relevant agencies that are not currently included in the Coordinated Framework for the Regulation of Biotechnology.

The introduction of novel genetic constructs intended to modify ecosystems increases the uncertainties that gene drives raise in ways that make robust assessment of their risks more critical, but also more difficult. Regulation will be needed that facilitates fundamental, applied, and translational research so that the potential harms and benefits of gene drives can be responsibly explored in laboratory and field studies.

Recommendation 8-5: Relevant agencies and decision making bodies will need to develop the capacity for robust assessment of a gene-drive modified organism's risks and uncertainties on a case-by-case basis that looks at the organism's intended function as well as the biological construct.

Recommendation 8-6: Regulatory agencies with oversight authority over genetic modification research should review risk assessment models and procedures to ensure that they capture the characteristics of gene drives, drawing upon multiple modelsand and integrating experts' comprehensive knowledge of practical conditions for gene drive research.

There is broad agreement on the importance of engaging affected communities and broader publics in decision making about activities involving gene drives. Mechanisms for public engagement and deliberation already exist within the relevant authorized agencies, but there is generally little clarity on how public engagement should feed into governance and a lack of consensus about best practices in this regard. This is due to at least two factors: first, because regulatory authority remains unclear, the availability of particular formal and customary mechanisms for public engagement also remain unclear; second, although the National Environmental Protection Act will in some cases require public input and afford opportunity for public comment, these mechanisms are an inadequate platform for the more robust forms of engagement discussed in Chapter 7.

The scientific community, including individual researchers, institutions, and funders, have an obligation to engage in conversations with policy makers about best practices to safeguard against unintentional or intentional misuse of gene-drive modified organisms. Safeguards will be aided by rigorous attention to confinement and containment protocols in laboratory and field tests; active awareness about the potential for misuse; and participation in education and training programs about the dual use potential of gene drive research. Governance mechanisms need to be in place to address questions about the biosecurity implications of gene drive research and consider develop mitigation strategies that are not dependent on the underlying technology.

Recommendation 8-7: Researcher institutions, regulators, and funders should collaborate to develop oversight structures to regularly review the state of gene drive science and its potential for misuse. Such reviews should also recommend or develop educational programs for researchers and members of the public about biosecurity concerns, the potential for dual-use research, responsible practices, and the funding of gene drive science.

Research on gene drives is global and likely to become even more so in the future. Responsible governance will need to be international and inclusive, with clearly-defined global regulatory frameworks, policies, and best practice standards for implementation. Low- and middle-income countries where gene-drive modified organisms may be employed will need to be involved in relevant governance, recognizing that many countries lack the capacity to develop a comprehensive regulatory scheme for gene drives from scratch. To cope with the unique aspects of gene drives, existing approaches to governance need to be adapted and combined for broad international use. Integrating new policy and law for gene drives into existing international governance frameworks will require attention to the values, experiences, and perspectives of people in many disparate nations. It is unlikely that a successful one-size-fits-all approach or

a single mechanism, such as regulation, policy, or professional codes alone, will be sufficient for appropriate international governance of gene-drive technology.

The most broad-ranging and widely accepted international governance system is the United Nations Convention on Biological Diversity, as implemented through the Cartagena and Nagoya Protocols. Many countries are now developing regulatory systems in response to the Cartagena Protocol. Many such systems are predicated on a strong precautionary, nearly preventative approach, which may restrict further gene drive research out of concern about gene drives' intrinsic ability to spread and persist in the environment. Given that the United States is not a Party to the Cartagena Protocol, it is a major gap in international governance that the United States does not have a clear policy for collaborating with other countries with divergent systems of governance, especially when such countries may, in fact, lack the capacity to assess the safety of gene drive research, undertake public engagement and societal dialogue, and maintain regulatory institutions. This gap is also significant because many sites for field testing, and ultimately environmental release of gene-drive modified organisms are likely to be outside of the United States.

> Recommendation 8-8: If field testing or environmental releases are expected to be conducted in other countries, United States funders and researchers should give careful consideration to the regulatory systems in place in those countries, their adequacy to control the development and release of gene-drive modified organisms, and the relevant community and other voices that will need to be considered in related governance.

In practice, a significant amount of field research on genetically-modified mosquitos operates under guidelines established by international organizations, such as the WHO, and by the research community itself. Although these guidelines provide a useful foundation for the establishment of guidelines for gene-drive modified organisms they have important gaps and may not address all of the unique aspects of gene drives or the range of potential organisms to be used. For example, guidelines may need to be adapted to align to local contexts in order to be implemented. Moreover, most guidelines are not tied explicitly to public oversight and implementation.

There is a need to reach international agreement on the adaptation of existing governance approaches in the United States and other countries to cope with the distinguishing features of gene drives, particularly their intentional persistence upon release to the environment.

> Recommendation 8-9: To ensure the long-term safety of human health and the environment, decision makers should consider a large toolbox of policies, including regulatory and non- regulatory mechanisms, for the rapidly developing field of gene drive research.

> Recommendation 8-10: Research institutions, regulators, and funders should revist international regulatory frameworks, national laws, non-governmental policy, and professional codes of conduct on research and the release of genetically modified organisms to determine whether and how they may be applied to the specific context of gene drive research, particularly with regard to site selection issues, capacity building for responsible and inclusive governance systems, scientific and post release surveillance, and stakeholder engagement.

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9

Gene Drives on the Horizon: Overarching Considerations

Scientists have studied what are now called gene drives for more than 50 years. But the development of a powerful genome editing tool in 2012, CRISPR\Cas9, led to the recent breakthroughs in gene drive research that build on that half century's worth of knowledge. Just prior to the beginning of this study and since the committee was first convened, scientists have published four proofs of concept—one yeast—one in fruit flies, and two in different species of mosquitoes—that demonstrate gene drives can be developed in the laboratory, at least in these organisms. Proposed applications for gene-drive modified organisms for basic research, conservation, agriculture, public health and other purposes will likely continue to expand as genome editing tools such as CRISPR become more refined. Gene-drive modified organisms are on the horizon.

Proof-of-concept in a few laboratory studies, however, does not lead to the immediate release of gene-drive modified organisms into the environment. Gene-drive modified organisms could bring very significant benefits, but to make sure that release does not cause more harm than good, more work remains to be done. Laboratory and field research is needed to refine CRISPR/Cas9 and other gene drive mechanisms, and to understand how gene drives might work under different environmental conditions and in a wide variety of other organisms. The considerable gaps in knowledge about potential off-target and non-target effects necessitate collaborative, multidisciplinary approaches to gene drive research, risk assessment, and public policy decisions for each proposed application of a gene-drive modified organism. Systems to share data and new knowledge will be needed as future gene-drive modified organisms are developed and prepared for release in confined field trials and into the environment

There is insufficient evidence available at this time to support the release of gene-drive modified organisms into the environment. However, the potential of gene drives for basic and applied research are significant and justify proceeding with laboratory research and highly-controlled field trials.

Recommendation 9-1: Funders of gene drive research should coordinate, and if feasible collaborate, to reduce gaps in knowledge not only about the molecular biology of gene drives, but also in other areas of fundamental and applied research that will be crucial to the responsible development and application of gene drive technology, including population genetics, evolutionary biology, ecosystem dynamics, modeling, ecological risk assessment, and public engagement.

Recommendation 9-2: Funders of gene drive research should establish open access, online repositories of data on gene drives as well as standard operating procedures for gene drive research to share knowledge, improve frameworks for ecological risk assessment, and guide research design and monitoring standards around the world.

The nature of gene drives—which are intended to spread select genetic elements into populations of living organisms—raises many ethical questions and presents a challenge for existing governance paradigms to identify and assess environmental and public health risks. In the United States and many other countries, governance of biotechnology, especially genetically modified organisms, is predicated on the

management of risk through confinement and containment. Gene drives do not fit well within the existing regulatory logic of confinement and containment because they are designed to spread a genotype through a population, making confinement and containment much more difficult (or even irrelevant) and the environmental changes introduced by release potentially irreversible. A phased testing pathway and robust ecological risk assessments are essential for navigating uncertainty and informing decisions around the development and application of gene-drive modified organisms.

Recommendation 9-3: The distinguishing characteristics of gene drives—including their intentional spread and the potential irreversibility of their environmental effects—should be used to frame the societal appraisal of the technology, and they should be considered in ecological risk assessment, public engagement, regulatory reform, and decision making.

Recommendation 9-4*:* Proposed field tests or environmental releases of gene-drive modified organisms should be subject to an ecological risk assessment and structured decision making processes. These processes should include modeling of off-target and non-target effects from the genome level through ecosystem level. When possible, empirical estimates of such variables as gene flow, population change, trophic interactions, and community dynamics should be developed as part of the models.

Public engagement can help to frame and define the risks of gene-drive modified organisms and provide input into practical decision making and policy development, but there are few avenues for such participation and insufficient guidance on how communities can and should take part. Without a defined process for public engagement and clear role for the public in assessment of gene drive technology, government accountability for related policy-making may be compromised, reducing the effectiveness of available governance mechanisms. Moreover, the goals of public engagement need to be clear, both to inform communities and stakeholders about gene drive research and to ensure their meaningful input into policy decisions. Ongoing and iterative public engagement can help to frame and define the relevant harms and benefits of gene-drive modified organisms, provide input into risk assessment and practical decision making, and align research and policy with public values. It will be particularly important for ecological risk assessment to reflect the values of relevant publics, and for the assessments to inform public policy decisions about emerging gene drive technologies, including comparisons with alternative strategies.

Recommendation 9-5: Governing authorities, including research institutions, funders, and regulators, should develop and maintain clear policies and mechanisms for how public engagement will factor into research, ecological risk assessments, and public policy decisions about gene drives. Defined mechanisms and avenues for such engagement should be built into the risk assessment and decision-making processes from the beginning.

Among the complex questions that arise for governance from gene drive research are how to select sites for field testing or environmental releases of gene-drive modified organisms, and who should be involved in making such decisions. Scientific and technical factors, including the presence of the target species and methods for confinement and containment, will need to be considered together with the values of the relevant publics that may be affected and their understanding of the risks, and the presence and capabilities of local governance bodies. Researchers will need to be able to engage with local communities, which may be particularly challenging in systems where democratic processes are not well established and power differentials may preclude some members of the public from such participation.

Recommendation 9-6: In selecting sites for field testing and environmental releases, researchers and funders should be guided by their professional judgement, the feasibility of risk assessment and community engagement, and the community's values and understanding of the balance of benefits and harms. In site selection, preference should be given to locations in countries with the existing

scientific capacity and governance frameworks to conduct and oversee the safe investigation of gene drives and development of gene-drive modified organisms.

A comprehensive approach to the development and governance of gene-drive modified organisms will need to go beyond considerations of public health and the environment, such as, but not limited to, the benefits of technological innovation, the implications of intellectual property, public engagement, and economics.

Gene editing is not a new endeavor. There are experts in the science and governance of gene editing whose experience could be applied to gene drive research with the aim of facilitating the exchange of knowledge.

Guidelines established by the World Health Organization (WHO) for research on genetically modified mosquitoes provide a useful foundation for the establishment of guidelines for gene-drive modified organisms. As WHO emphasizes for genetically modified mosquitoes, for example, the path for developing a gene-drive modified organism includes not only proof of efficacy, but also proof of acceptability and deliverability. Fundamental, applied, and translational laboratory and field research contribute to the proof of efficacy. Risk assessment, public engagement, and regulatory approval contribute to proof of acceptability. The cost-effectiveness of the technology versus alternative technologies may influence both acceptability and deliverability. In order to augment the deliverability of a gene-drive modified organism, a commitment to ongoing, long-term public engagement, and appropriate financing to support the monitoring of environmental releases are imperative.

Glossary

Accountability: Being answerable for one's actions or the ability to give an honest account of events and take responsibility for their consequences.

Adaptive management: An iterative decision-making process in which uncertainties are progressively resolved through monitoring of the system in question.

Allele: A variant form of a gene at a particular locus on a chromosome. Different alleles produce variation in inherited characteristics.

Asilomar: The 1975 Asilomar Conference on Recombinant DNA, convened to discuss the potential biohazards of recombinant DNA research, guidelines on safe laboratory practices, and the potential roles of regulation. The conference concluded that containment be made an essential aspect of experimental design, and that the effectiveness of containment practices matches the estimated risk of the particular experiment as closely as possible.

Attribute: a measurable characteristic of the ecological entity

Bayesian networks: Graphically depicted web of nodes that link cause and effect relationships using conditional probability to describe the interactions and to generate the probability outcome or outcomes.[1]

Biosafety: Policies and practices intended to prevent harm to the health or safety of human beings, other living organisms, or the environment, especially those pertaining to safe handling and containment of infectious agents.

Biosecurity: An integrated system of best scientific practices, environmental controls, and policy and regulation that identifies and manages risks of intentional misuse of technologies, particularly biological agents and processes, in ways that threaten public health or national security.

Biotechnology: A number of methods that endow new characteristics in an organism

Capacity building: The provision and promotion of education and practical training, particularly within low-resource and unskilled communities, often with respect to essential services.

Cartagena Protocol on Biosafety to the Convention on Biological Diversity: An international agreement that addresses the safe handling, transport, and use of living modified organisms resulting from modern biotechnology, with the aim of protecting biological diversity and human health. One hundred and seventy countries are signatories to the agreement, which took effect on 11 September 2003.

[1]Marcot, B.G., J.D. Steventon, G.D. Sutherland, and R.K. McCann. 2006. Guidelines for development and updating Bayesian belief networks applied to ecological modeling and conservation. Can. J. Forest Res. 36(12):3063–3074.

Community: a group of people who live near enough to a potential field trial or release site that they have tangible and immediate interest in the gene drive project.

Compliance: The act of following or obeying a rule or order, particularly with respect to governmental regulation.

Confinement: The use of ecological conditions or biological methods to prevent unintended or uncontrolled persistence of an organism in the environment.

Conservation: The protection and preservation of the natural environment or particular species, including the maintenance of habitats and genetic diversity.

Containment: The use of human-made or natural physical restrictions to prevent unintended or uncontrolled release of an organism into the environment.

CRISPR (Clustered regularly-interspaced short palindromic repeats): A naturally occurding mechanisms of immunity to viruses found in bacteria that involves identification and degradation of foreign DNA.

CRISPR/Cas9: a gene editing platform in which an endonuclease and a guide RNA are used to to introduce double strand breaks at a specified location within the genome.

Dual use potential: The potential for the findings from research intended for human benefit to be misused for intentionally harmful purposes.

Dual use research of concern (DURC): Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be misapplied to pose a significant threat to public health and safety, agricultural crops and other plants, animals, the environment, military equipment and supplies, or national security.[2]

Environmental assessment: a determination of whether a U.S. federal government decision to allow a specific action has the potential to cause significant environmental effects.

Environmental impact statement: a detailed document from proposed major U.S. federal agency actions that are expected to significantly affect the quality of the human environment.

Ecological risk assessment: the study and use of probabilistic decision-making tools to evaluate the likely benefits and harms of a proposed activity on the wellbeing of humans and environment, often under conditions of uncertainty.

Ecosystem services: The functions and products of ecosystems that contribute to human well-being.

Ecological entity: a species, population, habitat, or ecosystem characteristic or function.

Ecosystem: A dynamic biological system consisting of all of the organisms in a specific environment and the non-living features of the environment with which they interact.

Effect: a potential beneficial or harmful outcome.

[2]http://www.phe.gov/s3/dualuse/Pages/default.aspx

Engagement: Seeking and facilitating the sharing and exchange of knowledge, perspectives, and preferences between or among groups who often have differences in expertise, power, and values.

Endemic: A situation in which disease is present continuously at some level in an area.[4]

Endpoint: societal, human health, or environmental value that is to be managed or protected.

Epigenome: the physical factors affecting the expression of genes without affecting the actual DNA sequences of the genome.

Epistemic uncertainty: a lack of knowledge about determinate facts.

Field trial: An experiment designed to test a promising new product or process in a context similar to that in which the product or process is intended to be used.

Fitness: A description of the ability to survive and reproduce, equal to the long-term average contribution to the gene pool by individuals having a particular genotype or phenotype.

Fixation: 100% frequency of a gene.

Gene: a segment of DNA that serves as a basic unit of heredity.

Gene drive: A system of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced. Thus, the result of a gene drive is the preferential increast of a specific genotype that determines a specific phenotype from one generation to the next, and potentially throughout a population.

Gene editing: A technique that allows researchers to alter the DNA of organisms to insert, delete, or modify a gene or gene sequences to silence, enhance, or otherwise change an organism's specific genetic characteristics.

Gene flow: The transfer of genetic information from one population into another population (also called *gene migration*).

Genetic engineering: Introduction of DNA, RNA, or proteins manipulated by humans to effect a change in an organism's genome or epigenome.

Genetically modified: An organisms whose genotype has been altered, including alteration by genetic engineering and nongenetic engineering methods.

Genome: the complete sequence of DNA in an organism.

Genome editing: specific modifcationof an organisms' DNA to create mutations or introduce new alleles or new genes.

Genotype: An individual's genetic identity.

Germ line: A cellular lineage in sexually reproducing organisms that produces the gametes (eggs and sperm) which transmit genetic material to the next generation.

Gonotaxis: Biased movement toward the germline

Governance: The process of exercising oversight through traditions (standards of practice) or regulations by which individuals and communities are held accountable. Governance often involves such policy tools as professional standards of practice and codes of conduct; formal guidelines, agreements, and treaties; and legislation or other governmental regulation.

Homing endonuclease genes: A class of selfish

Homology-directed repair: A naturally occurring mechanism for repair of a DNA sequence in a cell that has a double strand break. This repair mechanism inserts a copy of the DNA sequence from a homologous chromosome or artificially added DNA with homologous sequence into the DNA that has the break as a template for the repair.

Horizontal gene transfer: Movement of genes between populations of otherwise distinct species.

Hybrid: The offspring of two plants or animals of different species or varieties.

Indigenous species: Species that occur naturally in a given geographic area or have evolved there without human intervention. Also called *native* species**.**

Institutional Animal Care and Use Committee: A multidisciplinary committee responsible for providing ethical review and oversight of research involving animal subjects, with the goals of protecting animal welfare and ensuring the quality of the science (also called an *animal welfare committee*).

Institutional Review Board (IRB) for the Protection of Human Subjects: A multidisciplinary committee responsible for providing ethical review and oversight of research involving human participants with the goal of protecting their welfare (also called an *ethics committee*, an *ethics review committee* or a *research ethics committee)*.

Invasive species: A non-indigenous (or non-native) species that disrupts and often replaces one or more indigenous species.

Linguistic uncertainty: ambiguities in the terminology used to describe concepts.

Meiotic drive: Any process which causes one male or female germ cell to be over- or under-represented during meiosis, and hence in the next generation.

Migration: The movement, often seasonal, of populations, groups, or of individuals across geographic space.

Mitigation: Actions, policies, and programs that serve to prevent, minimize, or compensate for disruption of the natural environment.

Monte Carlo method: a statistical analysis that relies on repeated sampling of probability distributions of model inputs to estimate the final probability distribution for each of the model outputs (also called *Monte Carlo experiments* or *Monte Carlo simulations*).[3]

Mutagenic chain reaction: A gene drive mechanisms to using CRISPR/cas9.

[3]Burmaster D.E., Anderson P.D. 1994. Principles of good practice for the use of Monte Carlo techniques in human health and ecological risk assessments. Risk Anal. Aug; 14(4):477-81.

Nature: The totality of the material universe, including the forces and processes that exist or occur independent of human action.

Non-target effect: A direct, unintended, short- or long-term consequence for one or more organisms *other than* the organism intended to be affected by an action or intervention. Concern about non-target effects typically centers around unforeseen harms to other species or environments, but non-target effects can also be neutral or beneficial.

Off-target effect: A direct, unintended, short- or long-term consequence of an intervention on an organism other than the intended effect on that organism.

Overreplication: Increased copies of a genetic element within and organism

Pathogen: A biological agent, such as a virus, bacterium, or parasite, that causes disease.

Phased testing pathway: A step-wise approach to guide the preparation for and conduct of research in the laboratory through environmental release.

Phenotype: The observable traits of an organism (i.e. how an organisms appears outwardly and physiologically).

Population: All of the individuals of a given species within a defined ecological area.

Population biology: The study of populations, including their natural history, size, migration, evolution, and extinction.

Population replacement: The use of genetic methods to change specific traits in an entire population.

Population suppression: Intentional reduction of the number or distribution of a population through physical, chemical, or biological means, particularly with pest species (also called *population reduction*).

Publics: groups who lack the direct connection to a project that stakeholders and communities have but nonetheless have interests, concerns, hopes, fears, and values that can contribute to democratic decision making.

Recombinant DNA (rDNA): Any novel DNA sequence created using genetic engineering.

Refractoriness: A condition in which an organism is intrinsically unable to support the development of a pathogen to an infective stage or to a point of sufficient abundance such that the organism cannot transmit disease.[4]

Responsible conduct of research: Commitment by researchers and their institutions to practices that sustain the integrity of science, particularly in the core areas of: conflict of interest; research with humans and animals and safe laboratory practices; mentor-trainee responsibilities and relationships; peer review; data acquisition, management, sharing and ownership; collaborative research; responsible authorship and publication; research misconduct and responding to allegations of misconduct; the scientist as a member of society; environmental and societal impacts of research; and other contemporary ethical issues in research.

[4]World Health Organization. "Guidance framework for testing of genetically modified mosquitoes." TDR news item. Available: www. who.int/tdr/news/2012/guidance_framework/en/index.

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Reversal drive: The currently theoretical process by which the effects of a gene drive are reversed, using either the process that triggered the original gene drive or another process as yet undeveloped.

Risk: The probability of an effect on a specific endpoint or set of endpoints due to a specific set of a stressor or stressors. An effect can be beneficial or harmful.

Risk assessment: The process by which all available evidence on the probability of effects is collected, evaluated, and interpreted to estimate the probability of the sum total of effects.

Risk communication: The process through which concerns about and tolerance of risk are articulated by stakeholders and the results of risk assessment and risk management are communicated to decision-makers and the public.

Risk management: The process of identifying and implementing measures expected to reduce risk to a tolerable level.

RNA interference (RNAi): A natural mechanisms found in nearly all organisms in which the levels of transcripts are reduced or suppressed.

Scientific community: A dynamic international, multidisciplinary network of scientists and scientific institutions including, for example, investigators, science educators, universities, research institutes, funding organizations, regulatory bodies, and publishers, united by their common commitment to the advancement of scientific knowledge through the use of critical, reproducible methods.

Selfish genetic elements: stretches of DNA that are certain to pass down from a parent organism to nearly all of its offspring.

Split gene drive: a research approach in which gene drive components (for example, Cas9, gRNA, and the donor template) are supplied separately to the organism.

Stakeholder: a person with a professional or personal interests sufficient to justify engagement, but may not have geographic proximity to a potential release site for a gene drive technology.

Standard operating procedures (SOPs): Written, step-wise instructions or descriptions of essential, routine practices, intended to ensure consistent and safe performance.

Sterile insect technique (SIT): a method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species. [5] Sterilization is typically carried out chemically or through exposure to radiation.

Stressor: any agent or actor with the potential to alter a component of the ecosystem.

Synthetic biology: The ability to develop novel traits or organisms using synthetic genes or by bringing together genes from multiple organisms. Also defined as the ability to generate novel traits or organisms using computational designed DNA or reagents that are not directly found in nature.

Target Product Profile: A strategic development process tool that uses set of criteria to pre-define ideal attributes of a candidate product and subsequent modifications to acceptance thresholds.

[5]FAO: http://www-naweb.iaea.org/nafa/ipc/sterile-insect-technique.html

Trait: A genetically determined characteristic or condition

Transcription Activator-Like Effector Nucleases (TALENs): a class of engineered restriction enzymes generated by the fusion of a transcription activator-like effector DNA-binding domain to a DNA-cleavage domain that can be used as a genome editing tool.

Transgene: Any gene transferred into an organism by genetic engineering

Transgenic organism: An organism into which one or more genetic sequences from another species or synthetic sequences have been introduced into its genome by genetic engineering.

Transposable element: small DNA segments can move from one part of the genome to another by excising themselves and randomly inserting elsewhere in the genome. Also called transposons or jumping genes.

Underdominance (also called heterozygous disadvantage): A condition in which the phenotypic expression of the heterozygote is less than that of either homozygote.

Values: Deeply held, complicated, sometimes evolving beliefts about what kinds of things—in humans' lives and the world at large—should be fortered, protected, or avoided.

Vector: An organism that spreads disease to other species by transmitting one or more pathogens rather than causing infection itself.

Wild-type: The collection of genotypes or alleles found in a natural population.

Wolbachia: A symbionts bacteria found in the cells of many invertebrates, including insects and nematodes that affect the reproductive biology of its hosts.

Zinc finger nucleases: a class of engineered restriction enzymes generated by the fusion of a zinc finger DNA-binding domain to a DNA-cleavage domain that can be used as a genome editing tool.

Acronyms

APHIS: Animal and Plant Health Inspection Service
Cas9: CRISPR associated protein 9
CERCLA: Comprehensive Environmental Response, Compensation, and Liability Act
CRISPR: Clustered regularly-interspaced short palindromic repeats
DNA: deoxyribonucleic acid
EA: environmental assessment
EIS: environmental impact statement
EPA: Environmental Protection Agency
FDA: Food and Drug Administration
FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act
GMM: genetically modified mosquito
GMO: genetically modified organism
gRNA: guide ribonucleic acid
HDR: homology directed repair
HEG: homing endonuclease genes
HGT: horizontal gene transfer
IACUC: Institutional Animal Care and Use Committee
IBCs: Institutional Biosafety Committees
LMO: living modified organism
Medea: Maternal-effect dominant embryonic arrest
NEPA: National Environmental Policy Act
NGS: next generation sequencing
NHEJ: non-homologous end joining
NIH: National Institutes of Health
NSABB: National Science Advisory Board for Biodefense
NRC: National Research Council
RAC: Recombinant DNA Advisory Committee
OSTP: Office of Science and Technology Policy
PAM: protospacer adjacent motif
RCRA: Resource Conservation and Recovery Act
RIDL: release of insects with dominant lethality
RNAi: RNA interference
rDNA: recombinant DNA
RRM: relative risk model
SD: Segregation Distorter
SIT: sterile insect technique
SOP: standard operating procedure
TALENs: Transcription activator-like effector nucleases
TPP: target product profile
TSCA: Toxic Substances Control Act
USDA: US Department of Agriculture
USFWS: US Fish and Wildlife Service
WHO: World Health Organization
ZFNs: zinc finger nucleases

Appendix A

Agenda for the Workshop on the Science, Ethics, and Governance Considerations for Gene Drive Research

Wednesday, October 28th, 2015

National Academy of Sciences Auditorium
2101 Constitution Avenue, NW
Washington, DC

8:00 am	Welcome and Introduction
	Purpose of the Gene Drive Study – Elizabeth Heitman, Study Co-chair, Vanderbilt University Workshop objectives and organization – James Collins, Study Co-chair, Arizona State University
8:10 – 10:00	Scientific Considerations
	Capabilities and tradeoffs of gene drive techniques – Austin Burt, Imperial College (8:10) Genome sequencing approaches and determining off-target effects of engineered nucleases: Shengdar Tsai, Massachusetts General Hospital (8:30) Understanding ecological and evolutionary conditions for gene flow Plants – Allison Snow, Ohio State University (8:50) Mosquitoes – Nora Besansky, University of Notre Dame (9:10) Discussion with the Committee (9:40)
10:10	Break
10:30 – 12:00	Responsible Conduct and Ethics
	Scientific integrity in research on emerging technologies – Francis Macrina, Virginia Commonwealth University (10:30) Ethics in science and governance of science – Bruce Jennings, Vanderbilt University (10:50) Do gene drives present novel ethical considerations? – Andrew Light & Jesse Kirkpatrick, George Mason University (11:10) Discussion with the Committee (11:30)
12:00	Break
1:00 – 2:40	Perspectives on Opportunities and Limitations in Low- and Middle-Income Countries
	Role of science in the development and governance of biosafety of biotechnology research in African countries – Diran Makinde, Africa Biosafety Network of Expertise (1:00)

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How interactions with communities influence vector control research directions and governance policies in Thailand – Wannapa Suwonkerd, Division of Vector-borne Disease Control, Ministry of Health (1:20)
Benefits and challenges for multi-country field trials of biotechnology in Latin America – Norma Padilla, Universidad de Valle de Guatemala (1:40)
Discussion with the Committee (2:10)

2:40 Break

3:00 – 4:40 Scales of Governance

International mechanisms to govern biotechnology – David Wirth, Boston College (3:00)
U.S. governance of biotechnology – Megan Palmer, Stanford University (3:20)
Institutional governing policies – Zach Adelman, Virginia Tech (3:40)
Discussion with the Committee (4:10)

4:40 Break

5:00 Public Comment Period

6:00 Adjourn

Appendix B

List of Gene Drive Webinars

1. **Gene Drive Research in Different Organisms,** October 15, 2015
   Speakers:
   Fred Gould, North Carolina State University – *General Overview*
   Zachary Adelman, Virginia Tech - *Gene Drives in Mosquitoes: Disease Vector Control*
   John Godwin, North Carolina State University – *Gene Drives in Rodents for Invasive Species Control*
   Weblink: http://nas-sites.org/gene-drives/2015/10/02/webinar-gene-drive-research-in-different-organisms/

2. **Current Status and Next Directions for Basic Research on Gene Drives,** October 21, 2015
   Speakers:
   Ethan Bier & Valentino Gantz, University of California, San Diego – *Gene Drives: Finding a Balance between Safety and Implementation*
   Weblink: http://nas-sites.org/gene-drives/2015/10/02/webinar-current-status-and-next-directions-for-basic-research-on-gene-drives/

3. **Considerations for Commercial Applications of Gene Drives,** November 2, 2015
   Speaker:
   Luke Alphey, The Pirbright Institute
   Weblink: http://nas-sites.org/gene-drives/2015/10/03/webinar-commercialapps/

4. **Key Principles and Considerations for Risk Assessment of Gene Drive Research and Applications**, November 5, 2015
   Speakers:
   Katherine von Stackleberg, Harvard University – *Risk Assessment to Support Decision Making*
   Bruce K. Hope (retired) – *Three Take Home Messages about Risk Assessment*
   Weblink: http://nas-sites.org/gene-drives/2015/10/04/webinar-risk-assessment/

5. **Biosecurity Implications of Gene Drive Research,** November 19, 2015
   Speakers:
   Edward You, Federal Bureau of Investigations – *General Considerations for Biosecurity*
   Jacqueline Fletcher, Oklahoma State University – *Implications of Gene Drives for Agricultural Security*
   Amesh Adalja, University of Pittsburg Medical Center – *Potential for the Use of Gene Drives in Entomological Warfare*
   Weblink: http://nas-sites.org/gene-drives/2015/10/07/implications-of-gene-drive-research-on-biosecurity-webinar/

6. **Species Interaction Dynamics and Ecological Community Structures in the Context of Gene Drives,** November 20, 2015
   Speakers:
   David Lodge, University of Notre Dame – *Invasions and Extinctions of Species*
   George Roderick, University of California, Berkeley – *Lessons from Islands*
   Weblink: http://nas-sites.org/gene-drives/2015/10/08/webinar-interaction-dynamics/

7. **U.S. Regulation of Biotechnology,** December 9, 2015
   Speakers:
   Sarah R. Carter, J. Craig Venter Institute – *An Overview of the Landscape of U.S. Regulations of Biotechnology*
   Larisa Rudenko, U.S. Food and Drug Administration – *Regulation of GE Animals at the U.S. Food and Drug Administration: FD&C Act and NEPA*
   Alan Pearson, U.S. Department of Agriculture – *Regulation of Agricultural Biotechnology by USDA-APHIS.*
   Weblink: http://nas-sites.org/gene-drives/2015/11/14/webinar-us-regulations/

8. **Containment Guidelines for Gene Drive Research,** December 15, 2015
   Speakers:
   Mark Benedict, Centers for Disease Control and Prevention, Atlanta – *Practicalities of Insects Containment in Multi-use Laboratories*
   Steve Strauss, Oregon State University, Corvallis – *Lessons Learned from Regulated Field Trials of Transgenic Trees and Implications for Potential Gene-drive Applications*
   Weblink: http://nas-sites.org/gene-drives/2015/12/01/webinar-containment/

9. **Field Research with Modified Organisms,** December 15, 2015
   Speakers:
   Scott O'Neil, Monash University, Melbourne, Australia – *Field Release of Wolbachia Infected Mosquitoes to Control Dengue Virus Transmission*
   Danilo Carvalho, International Atomic Energy Agency, Vienna – *Lessons Learned from Sustained Field Release of Transgenic "Sterile" Male Mosquitoes in Brazil*
   John Marshall, University of California, Berkeley – *Genetically Modified Mosquito Strategies and Disease Modeling to Control Malaria in Sub-Saharan Africa*
   Weblink: http://nas-sites.org/gene-drives/2015/11/24/webinar-field-research-with-modified-organisms/

10. **Perspectives on Environmental Benefits and Hazards of Gene Drive Research,** December 17, 2015
    Speakers:
    Owain Edwards, Commonwealth Scientific and Industrial Research Organization – *Ecological Consequences of Gene Drives: Addressing the Uncertainties*
    Kent Redford, Archipelago Consulting – *Synthetic nature and the future of conservation*
    Weblink: http://nas-sites.org/gene-drives/2015/12/08/environmentalperspective/

11. **Building International Capacity for Research and Technology Assessment of Gene Drives,** January 5, 2016
    Speakers:
    Genya Dana, US State Department – *International Biotechnology Policy and Research Capacity Building*
    Cliff Goodman, The Lewin Group – *Building Capacity for Technology Assessment*
    Weblink: http://nas-sites.org/gene-drives/2015/12/30/webinar-capacity-building/

Appendix C

Mosquito Control Strategies

A list of mosquito strategies that are in use or in development are listed in Table C-1. As noted in Chapter 3 of this report, many of the strategies in use are labor intensive, reactive, and are losing their effectiveness if they work at all (Achee et al., 2015).

TABLE C-1 Some Mosquito Control Strategies in Use or in Development

Name	Primary Outcome(s)	Key Advantage(s)	Primary Challenge(s)	Select References
Strategies in Use[a]				
Long-Lasting Insecticidal Nets (LLINs)	Bite prevention; population reduction	Community impact	Insecticide resistance; daytime biting vectors	Smith Gueye et al., 2016 Briet and Penny, 2013
Indoor Residual Spray (IRS)	Population reduction	Reduction of household insect pests	Insecticide resistance; outdoor vector resting	Smith Gueye et al., 2016 Yakob et al., 2011 Zhou et al., 2010
ULV/Space-spraying	Population reduction	Highly visible by community	Insecticide resistance; delivery inside homes	Bonds, 2012 Esu et al., 2010
Larvicides and larval source management	Population reduction	Minimal interference with end-user lifestyle	Delivery at adequate scale; insecticide resistance	Fillinger et al., 2011 Imbahale et al., 2012 Tusting et al., 2013
Biocontrol	Population reduction	Resistance independent	Delivery at adequate scale	Majambere et al., 2007 Scholte et al., 2006
Gravid ovitraps	Population reduction	Shared health ownership with community	Delivery at adequate scale; maintenance	Eiras et al., 2014 Mackay et al., 2013
Personal repellents (DEET)	Bite protection	Use for outdoor biting; niche transmission settings (forest)	End-user compliance	Debboun and Strickman., 2013 Katz et al., 2008
Strategies in Development				
Attractant-Lethal Sugar Baits (ATSB)	Population reduction	Targets both male and female mosquitoes	Effect on non-target organisms	Beier et al., 2012 Muller et al., 2010
Push-Pull	Bite prevention; population reduction	Targets indoor and outside biting	Cost of delivery and maintenance (trap)	Menger et al., 2015 Menger et al., 2014 Wagman et al., 2015
Spatial Repellents	Bite prevention	Continual protection in treated space	Cost of delivery and replacement	Hill et al., 2014 Achee et al., 2012 Syafruddin et al., 2014
Wolbachia	Population reduction; replacement	Introduced symbiont in mosquitoes	Infrastructure to maintain colonies; multiple releases	WHO, 2010 Bian et al., 2010 McMeniman et al., 2009 Hoffmann et al., 2011 Iturbe-Ormaetxe et al., 2011 Moreira et al., 2009 Dobson et al., 2002

(Continued)

TABLE C-1 Continued

Name	Primary Outcome(s)	Key Advantage(s)	Primary Challenge(s)	Select References
Release of Insects with Dominant Lethality (RIDL)	Population reduction	Release of non-biting males	Infrastructure to maintain colonies; multiple releases	WHO, 2010 Alphey et al., 2010 Atkinson et al., 2007 Phuc et al., 2007
Pyriproxyfen (PPF)	Population reduction	Target of cryptic habitats	Density-dependent phenomena	Devine and Killeen, 2010 Harris et al., 2013 Koama et al., 2015 Lwetoijera et al., 2014

[a]Recommended by the World Health Organization.
Source: Modified from Achee et al., 2015.

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Appendix D

Rodent Control Strategies

A comprehensive list of rodent control strategies that are in use or in development are listed in Table D-1. As noted in Chapter 3 of this report, many of the strategies in use are labor-intensive, expensive, and have limited effectiveness.

Summary of current technology for rodent control (adapted from NCSU website)[1].

TABLE D-1 Some Rodent Control Strategies in Use or in Development

Name	Primary Outcome(s)	Key Advantage(s)	Primary Challenge(s)	Select References
Strategies in Use				
Toxicants (coagulants such as Brodifucoum)	Species elimination	Very effective for use in rats but not so for mice Are odorless and tasteless so rodents can't evade them	Low number of feedings required in order to prevent avoidance of them Animal welfare issue (leads to painful death) Secondary, non-target effects (ecological and animal welfare concerns) lead to question of feasibility	Williams, 2013 Mensching and Volmer, 2008 Meerburg et al., 2008 Thomas and Taylor, 2002
Mechanical (kill and live traps)	Species elimination or translocation	Little to no risk to human health or environment, no toxins released to ecosystem	Inability to discriminate between target and non-target species Animal welfare issues	Witmer and Jojola, 2006 Lorvelec and Pascal, 2005 Hygnstrom and Virchow, 1992
Biological controls	Species elimination	Easy to identify, potential decreased risk to humans Sometimes species-specific in their efficacy	Biological controls	Garden, 2005 Saunders et al., 2010 Weber, 2010
No action (i.e., species remains in the environment)	N/A	No cost	Damage to biodiversity; other (ecological outcomes)	
Strategies in Development				
RNAi, immunocontraception	Species elimination or reduction	Species-specific, lowers reproductive capacity (autoimmune infertility)	Technical challenges associated with the design and delivery of treatment, target population at correct time and in large numbers. Anti-fertility technique may not be effective if these animals attempt to mate with wildtype animals.	Heath et al., 2014 Xue et al., 2012 Jacob et al., 2008 Biotechnology Australia (2001) www.biotechnology.gov.au Chambers et al., 1999
Transgenic approaches	Species elimination or reduction	Species-specific; induces sex lethality or sex reversal	Would require multiple releases of modified males; may not be scalable	Campbell et al., 2015 Gemmell et al., 2013 Bax and Thresher, 2009 McLaren and Burgoyne, 1983

[1]https://research.ncsu.edu/islandmice/what-has-been-done/history-of-rodent-eradications/.

Appendix D

Rodenticides

First-generation compounds, such as warfarin, must be administered in high concentrations over multiple doses, and thus have now been replaced by second-generation compounds, such as the odorless and tasteless toxicant Brodifucoum (Thomas and Taylor, 2002; Mensching and Volmer, 2008). If the terrain affects the ability to successfully apply the chemicals, then rodents in these areas may not be treated. Mechanical methods such as trapping are not considered feasible but can be used in conjunction with other methods.

Traps

Mechanical traps are considered by some to be more humane than rodenticides. Collectively, these mechanical methods cannot discriminate between target and non-target organisms (Lorvelec and Pascal, 2005, Biological Invasions), and so similar issues are raised to the use of chemical toxicants.

Biological Controls

Biological controls of invasive rodents include predators, parasites, or other disease-causing agents that act by recapitulating the factors that would normally limit the population. One of the considerations in using this method is whether the introduction of such an agent would itself become invasive given its placement in an environment that is not its own. Several unsuccessful examples of the deployment of this method can be found in the literature, such as the introduction of rabbits into Australia in the late 1800s (Garden, 2005), means to control their subsequent substantive, and unexpected, population growth (Saunders et al., 2010), or the introduction of the cane toad to control agricultural pests of Australian sugar cane (Weber, 2012). The cost of this type of intervention will vary depending upon the organism of interest and the biological control agent being introduced.

Genetic Engineering Strategies in Development

One method being explored takes advantage of the process of RNA interference (RNAi), in which double-stranded RNAs that target endogenous RNAs essential for the life of the rodent would be introduced to the rodent in an analogous fashion to that observed currently for agricultural pests (Xue et al., 2012). Technical issues associated with this technique include delivery of double-stranded RNAs, their inherent stability and thus persistence of inhibition, the concentration required to effect species eradication, mechanism of spread, and potential biosafety risks. Proof-of-concept using RNAi as a toxicant has been demonstrated, however, with sea lampreys (Heath et al., 2014), and delivery of small interfering RNAs has been shown to be possible in mice (He et al., 2013). Another approach is autoimmune infertility, in which a virus is used to express proteins that elicit an immune response targeting the fertilization process, thus preventing formation of the zygote (Chambers et al., 1999). This technique would achieve population reduction, but challenges still remain with respect to administration of the virus at the appropriate life cycle time of the rodent, the number of rodents that would be required to be infected (Jacob et al., 2008), and the need to ensure that infected rodents mate with one another as opposed to untreated rodents.

Another line of research involves a genetic approach in which rodents could carry transgenes that, upon mating to the invasive population, do not produce any progeny (e.g., lethality) or cause the female offspring to develop as males (sex-reversal) (Gemmell et al., 2013; Bax and Thresher, 2009; McLaren and Burgoyne, 1983). This method, however, will likely require multiple releases of transgenic males and may not be scalable (Campbell et al., 2015). Finally, in some instances it may not be possible to eradicate an invasive rodent population, due to the high cost involved, the location and topography of the land area under investigation, the presence of humans, or risks posed to the ecosystem.

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Appendix E

A Brief History of Ecological Risk Assessment

The field and practice of ecological risk assessment has evolved substantially over more than a quarter century. The field of risk assessment, whose origin in the United States is summarized in the 1983 NRC report *Risk Assessment in the Federal Government: Managing the Process* (widely known as "the Red Book"), preceded the emergence of ecological risk assessment. In the beginning, the main stressors of interest were chemicals and the endpoints of interest were cancer and human health; ecological effects were not a part of this initial formulation. By the late 1980s, there was growing interest in ecological processes and effects, prompting EPA to begin preliminary work on guidelines for risk assessment focused on ecological effects of stressors. In the early 1990s, EPA generated a framework and guidance documents for the conduct of ecological risk assessment (EPA 1992, 1998), and similar guidance documents were developed in Europe, Canada, and Australia.

In 2006, the Ecological Processes and Effect Committee of the EPA Science Advisory Board held a workshop on the current and future practice of ecological risk assessment that led to four important publications: Suter (2008) summarized the history of the development of ecological risk assessment from the mid-1980s to the mid-2000s; Barnthouse (2008) outlined the strengths of ecological risk assessment; Kapustka (2008) detailed some of its limitations as they stood in the mid-2000s; and Dale et al. (2008) provided a list of conclusions and recommendations for improving ecological risk assessment and its use in the decision-making process. The workshop and the subsequent papers brought to the forefront key aspects of ecological risk assessment and ways to improve it. For example, Dale et al. highlighted the critical importance of communication with decision makers and stakeholders during the development of endpoints and management questions, and recommended that a peer review be conducted at the problem-formulation stage. Having appropriate endpoints and management questions is essential to the ability to accurately describe cause-effect pathways and inform decision making. The workshop also underscored the importance of analyzing and reducing uncertainty to increase the predictive power of the risk assessment. Dale et al. suggested that risk assessment and monitoring programs should be better integrated, and recommended post-cleanup assessments to facilitate this. The workshop also called for methods to quantify the weight-of-evidence process, and recognized that ecological risk assessments should include the effects of chemical and non-chemical stressors at various organismal and ecological levels of organization and spatial scales. Finally, the workshop identified the need to develop methods to estimate cumulative risk assessments, together with techniques to deal with the reality that a number of stressors exist in the environment, not just the one of current regulatory interest. Several of these recommendations were echoed in the 2009 NRC report *Science and Decisions: Advancing Risk Assessment*. The report, a comprehensive review of EPA's human and ecological assessment process, remains an important milestone in assessing contemporary risk assessment and structured decision making within EPA.

Recently, Greenberg et al. (2015) reflected on contemporary practice in risk assessment since the NRC's *Red Book* report. Concluding that many of the *Red Book's* recommendations still hold, the authors noted that the view of risk assessment presented in the report has proved applicable to a much broader variety of circumstances than it was explicitly intended to address. For example, although the *Red Book* discussed risk assessment in the context of chemical exposures and a narrow set of effects, the process has been used for engineering, ecological effects, and other fields, and the oil, rail, chemical, aerospace, and

medical fields have adopted risk assessment as a standard practice. However, the application of risk assessment in the context of ecology has been somewhat more limited, perhaps reflecting a lack of understanding by risk assessors that ecological systems are nonlinear, complex, uncertain, and dynamic, and that outcomes are determined by multiple sources of stress. A symptom of this limited vision, for example, may be the late recognition of the importance of climate change (Landis et al., 2013) in evaluating risk to large-scale systems.

The following sections discuss the evolution of key aspects of ecological risk assessment, as well as specific applications, that may help to inform risk assessment approaches for gene-drive modified organisms.

CUMULATIVE RISK ASSESSMENT

Chapter 7 of the 2009 NRC report *Science and Decisions: Advancing Risk Assessment*, titled Implementing Cumulative Risk Assessment, is especially pertinent to the risk assessment of gene-drive modified organisms, because it defines cumulative risk assessment and elucidates the importance of expanding risk assessment beyond a narrow focus on a specific stressor.

Two methods for performing cumulative risk assessment have been described. An approach known as *stressor-based cumulative risk assessment* (Menzie et al. 2007) focuses on integrating multiple stressors, management options, and endpoints into a conceptual model that is used as the basis of risk assessment. The method uses the conceptual model to evaluate the likely stressors, their sources, and combinations of interactions that may occur. In the four-step assessment process outlined by Menzie et al., steps 3 and 4 focus on the range of management options. The NRC report *Science and Decisions: Advancing Risk Assessment* (2009) proposed modifying this approach to reduce the number of interactions to be considered, given that many of the original considerations included in the method would not be amenable to management and therefore are less pertinent to the risk assessment process.

The other method of cumulative risk assessment is the *relative risk model* (RRM) proposed by Landis and Wiegers (1997). This approach uses a ranking system to combine the interactions between multiple sources, stressors, habitats, and effects to estimate impacts to ecological structures. Wiegers et al. (1998) applied this approach to the Exxon Valdez oil spill and its effects on Port Valdez, Alaska. Since then, assessments using the RRM have been completed for a variety of stressors and combinations of stressors including contaminants, disease, environmental parameters, non-indigenous species, and the evaluation of landscapes (Walker et al., 2001; Moraes et al., 2002; Hayes and Landis, 2004, Colnar and Landis, 2007; Bartolo et al., 2012; Hines and Landis, 2014; Ayre et al., 2014; Allen et al., 2015; Kanwar et al., 2015; Heenkenda and Bartolo, 2015). Ayre and Landis (2012) also demonstrated how the RRM could be applied to management options. Since the early 2000s, Monte Carlo sampling has been used to describe uncertainty and to identify those variables that have the biggest impact on risk (Landis and Wiegers, 2005).

APPLYING ECOLOGICAL RISK ASSESSMENT TO INVASIVE SPECIES

In many ways, the release of gene-drive modified organisms is similar to the movement of invasive species. The early application of ecological risk assessment for the evaluation of invasive species was described in Andersen et al. (2004a, 2004b), which stemmed from a workshop convened to bridge the gap between risk assessment as described in EPA's 1998 guidance and the evaluation of invasive species. Several points from Anderson et al. (2004b) are especially relevant to gene drive research:

- There is a need for a conceptual basis for identifying assessment endpoints, determining the appropriate spatial and temporal scales, and describing the complexity of the resulting model.
- There is a need for guidance regarding the use of analytic tools; Bayesian or resampling (Monte Carlo) approaches are preferred.

- Multi-scale, spatially-explicit support systems, such as GIS mapping data tied to landscape population models, enhance credibility and make clear the trade-offs and the costs of inaction, thus supporting better decision making.
- The basic framework for an invasive species risk assessment (outlined in Figure 6-1 of this report) follows a source−exposure−habitat−effects−impact structure. For invasive species, the source is the native range of the species, exposure is the transport, habitat is the port of entry, and effects would describe the demography of the invasive species. The impact refers to the effects that are due to the presence of an invasive population.

Landis (2004) expanded the original framework proposed by Andersen et al. (2004b) into a generic conceptual model for invasive species following the basic formula previously used for the relative risk model (Landis and Wiegers, 1997). Modifications address the specific factors important to dealing with invasive species, and propose a basic computational framework for calculation using a Monte Carlo approach. Colnar and Landis (2007) used this framework to detail the risk posed by the invasion of the European Green Crab in the Northern Puget Sound at Cherry Point, Washington. The conceptual model was spatially specific and included multiple stressors and multiple endpoints. For some endpoints, the European Green Crab provided a negative risk (i.e., a benefit), for example, because it represented an additional food resource for native animal populations. However, the invading crab was determined to be detrimental in regard to other endpoints, such as those related to effects on native crab species and habitat.

Herring et al. (2015) applied the same basic structure but used Bayesian networks to assess risks posed by invasive species in the Padilla Bay National Estuarine Research Reserve in Anacortes, Washington. Puget Sound is already colonized by a large number of invasives and serves as a source of input to Padilla Bay. Evaluating potential mitigation strategies, the case study found that the treatment of ballast water at two nearby refineries would not substantially reduce the risk due to invasive species.

RECENT DEVELOPMENTS IN ECOLOGICAL RISK ASSESSMENT

A three-day workshop held at the Sydney Institute of Marine Sciences in Sydney, Australia in September 2014 provided a forum for examining the state of ecological risk assessment, identifying limitations of current practice, and proposing criteria for future assessments, with a focus on evaluating risk in the context of multiple stressors at large spatial scales as integrated into an adaptive management scheme. Van den Brink et al. (2016) presented findings and recommendations from the workshop, which are summarized here.

A major limitation identified by workshop attendees was that ecological risk assessments have been focused on single stressors affecting only a few receptors over relatively small spatial scales. However, many systems are affected by numerous abiotic and biotic factors, including disruption of the landscape by development, the introduction of non-native species, and the use of multiple agricultural chemicals. If only the stressor of primary interest is included in the risk assessment, the assessment will overlook interactions with other stressors and the risk will be presented out of context. In addition, ecological risk assessments often have not appropriately accounted for the fact that the intensity of the stressor will vary by location and over time. Indirect effects may also play a critical role and in some cases can be more influential that direct effect on the endpoints. Specific limitations of many ecological risk assessments include:

- Inherent limitations stemming from a lack of knowledge, as well as contrived limitations stemming from outdated guidance and regulations on conducting ecological risk assessments.
- A lack of useful data needed to answer questions specific to the risk assessment question, especially a lack of site-specific data.
- The reductionist process typical of ecological risk assessments, which extrapolates from organisms to ecosystems and from small-scale to large-scale systems, has not been tested adequately.

- The current process lacks transparency and relevant information is difficult to communicate to stakeholders.
- Little is known about how the composition of a community affects the response of organisms or ecosystems to stressors.
- Without effective diagnostic tools to link effects observed in the environment to the stressors' mode or modes of action, it has been difficult to determine cause and effect relationships.
- Too often ecological risk assessments use metrics that result in a simplified scorecard that does not take into account the interactions of the stressors, the organisms, and the effects of the landscape; as a result, these assessments can present a misleading picture of the true impacts.

Van den Brink et al. (2016) recognized the importance of ecological risk assessment to the adaptive management process as originally proposed by Wyant et al. (1995), which explicitly incorporates social goals. Social considerations and values, as expressed by the engagement and governance process, set the management goals and limits on resources and are factored into decision making. Ideally, the science of risk assessment estimates risk, evaluates management options, lists the critical variables to be monitored, and then re-evaluates the system.

Van den Brink et al. (2016) listed 11 practical steps for improving future ecological risk assessments:

1) Build a digital map of the study site that includes land use, topography, regulatory jurisdictions, and the locations of sources, stressors, habitats, and endpoints. This map becomes the framework for the risk assessment.

2) Establish *a priori* the cultural and protection goals that will determine the success of the assessment and decision-making process.

3) Determine the interactions among the species and the ecological processes and functions that will be affected by the stressors. Models are recommended as the tool for codifying these interactions when building the risk assessment.

4) Map out regions in the landscape that have similar land uses, stressors, and management goals. These regions are useful in describing the distribution of risk across the study region.

5) Build a list of management activities, ranging from simple nutrient reduction to major civil engineering activities such as building cofferdams.

6) Construct a conceptual model that reflects the sources of stressors, the stressors, habitats, the expected effects, and the impacts to the system under investigation.

7) Use the conceptual model to organize all of the information that will inform the cause-effect modeling. This activity will help build the necessary model but also is a communication tool for decision makers and stakeholders.

8) Use the best tools to describe cause-effect relationships in a probabilistic manner.

9) Transform the cause-effect model into a quantitative structure using approaches that incorporate the dual deterministic and probabilistic nature of ecosystems. This recommendation explicitly recognizes that there are both deterministic and probabilistic features of ecological interactions.

10) Use large datasets and modern statistical tools to improve the accuracy of the predictions and to better quantify or reduce the uncertainty of the risk assessment process. These tools are innately probabilistic and also are robust in providing evidence for cause-effect interactions.

11) Employ ecological risk assessment as part of an adaptive management framework as suggested by Wyant et al. (1995).

Applying Ecological Risk Assessment to Genetically Modified Organisms

The development and release of genetically modified organisms brings many of the same ecological considerations as the development and potential release of gene-drive modified organisms. As such, a review of frameworks and examples of assessments that have been applied to genetically modified organisms provides useful context.

Tiedge et al. (1989) published an early summary of the potential hazards and effects of genetically modified organisms. The authors recommended that the assessment of genetically modified organisms should be based on phenotypic traits rather than on how the organism was created. They identified several factors that could be useful in estimating the effects of genetically modified organisms on the environment, including:

- Survival and reproduction of the genetically modified organism;
- Interactions between the organism and the ecosystem in which it is released;
- Effects on the structure and function of ecosystems;
- Changes in the fitness of the modified organism;
- Genetic transfer of the introduced sequence by hybridization, conjugation, transduction, or transformation; and
- Potential displacement of native species.

Recognizing the potential for genetically modified organisms to cross national boundaries, the authors suggested the need to establish a means for international coordination regarding the regulation of biotechnology.

Many of the points made by Tiedge et al. were reiterated by Snow et al. (2005) in a position paper from the Ecological Society of America. The paper, which uses an alternative term for genetically modified organisms, genetically *engineered* organisms (GEO), included the following conclusions and recommendations:

- GEOs should be designed to reduce environmental risks.
- More extensive studies of the environmental benefits and risks associated with GEOs are needed; effects should be evaluated relative to appropriate baseline scenarios.
- Environmental release of GEOs should be prevented if scientific knowledge about possible risks is clearly inadequate.
- In some cases, post-release monitoring will be needed to identify, manage, and mitigate environmental risks.
- Science-based regulation should subject all transgenic organisms to a similar risk assessment framework and should incorporate a cautious approach, recognizing that many environmental effects are GEO- and site-specific.
- Ecologists, agricultural scientists, molecular biologists, and others need broader training and wider collaboration to address these recommendations.

The paper is an excellent compendium of the types of genetically modified organisms, their potential uses, and the possible effects. The paper also discusses ecological risk assessment and uncertainty, though not in a concrete fashion.

Another landmark paper in the discussion of ecological effects of genetic modification is Burt (2003), which describes the use of site-specific selfish genes as tools to control natural populations. The paper discusses the probability of horizontal gene transfer and describes nuances and effects of the homing endonuclease gene, including how frequently it changes over time and its relationship to the fitness of the population. The paper's population models are idealized, and appear to assume that an equilibrium state can be reached. These models are similar to those described in the population genetics section of the current report and draw from a framework developed originally by Hartl (1970). The paper is significant in that it covers some key considerations that inform the construction of a conceptual model and notes that the estimations of frequency change as a construct moves through a population.

By the mid-2000s, it had become apparent that the traits introduced into genetically modified plants could move to wild plants of the same species or to closely-related organisms. For example, it has been documented that the CP4 EPSPS marker, which confers resistance to glyphosphate herbicide, transferred from creeping bentgrass (*Agrostis stolonifera*) to sentinel plants of *A. stolonifera* and other *Agrostis* plants in the landscape; that transgenic herbicide-resistant *Agrostis stolonifera* had become established in areas downwind of cultivated areas, suggesting a pollen-mediated dispersal; and that *Agrostis* hybrids were fertile and stable (Watrud, et al. 2004; Reichman et al., 2006; Kausch et al., 2010). Such examples may be useful in understanding the potential for gene flow between gene-drive modified organisms and other organisms.

Tiered approaches to assess effects have long been part of environmental toxicology and other fields. Raybould and Cooper (2005) used a series of tiered tests to evaluate the risk of changes in hybrids between virus-resistant transgenic *Brassica napus* and wild relatives. The authors proposed three tiers: Tier I tests for hybrid production using laboratory experiments and hand pollination; Tier II looks for spontaneous hybrids in a laboratory or field setting; and Tier III searches for naturally occurring hybridization. The authors presented case studies to demonstrate the prediction of risk using the tiered approach. However, the analysis is a comparison of exposure to an effect threshold to determine a risk quotient; as such, the description of the risk assessment and uncertainty is not quantitative, and the analysis lacks a clear conceptual model.

Wolt et al. (2010) proposed a problem formulation process that is reminiscent of the framework described in EPA's 1998 guidance for ecological risk assessments, though the terminology used is somewhat confusing. For example, the authors state that identifying "risks of greatest relevance" is at the core of the problem formation process; however, it is not clear whether "risk" is intended to be synonymous with hazard (as is the common-language interpretation), as a probabilistic technical term, or in a discipline-specific way. In addition, uncertainty is defined as "a form or source of doubt," which is different from its definition used in this report. Although specific to the risk assessment of genetically modified organisms, it appears that many of the authors' key points had been superseded by earlier research.

Selecting appropriate test species is an important task in ecological risk assessment. In a review of the criteria for selecting arthropod species for testing to derive ecological risks from crops genetically modified for insect resistance, Romeis et al. (2013) identify test organisms that have been used for regulatory risk assessment. The authors recommend selecting species that are relevant and avoiding superfluous data that could distract the attention of risk assessors from more serious risks. However, the authors use the term "risk" as synonymous with hazard in this work. In addition, risk needs to be estimated before a comparative ranking of risk can be accomplished.

LEARNING FROM ASSESSMENTS UNDER NEPA

As discussed in Chapter 6 of this report, alterations to the environment are often assessed under the EA and EIS process in compliance with NEPA. Some of these assessments can provide insight into the types of environmental considerations to be included when the release of a genetically modified organism

is planned as part of environmental management. APHIS (2008), for example, is an environmental impact assessment for the use of genetically engineered insects as part of a pest control program. As an environmental impact statement, the report does not fit the probabilistic cause-effect structure of a risk assessment. However, the report does contain information that would be useful in a problem formulation process. Section IC of the report describes a range of potential scenarios and maps the locations of rearing sites and program activities. Section III, Affected Environment, provides a detailed listing of the range of environments where the genetically modified organisms would be used. Section IIIC discusses the affected environment, including human health and non-target species. In a risk assessment, many of these lists would correspond to culturally important endpoints, whether they are cultural resources, listed species, visual resources, domestic animals, critical habitats, or wild plants or animals.

Another illustrative environmental impact assessment is APHIS (2014), which focuses on a field release of the genetically modified diamondback moth. Similar to APHIS (2008), this report does not have the probabilistic cause-effect structure found in an ecological risk assessment, but could serve as a useful resource for constructing a conceptual model and computational framework for a risk assessment of a gene-drive modified organism.

RISK ASSESSMENT FOR STERILE MODIFIED MOSQUITOES

The risk assessment conducted by Hayes et al. (2015) for a hypothetical release of a modified sterile male mosquito provides perhaps the clearest parallels to gene-drive modified organisms. The scenario features the escape of modified sterile male mosquitoes from a research facility in a setting where wild-type mosquitoes of the same species are present in the environment. There is no published experimental or field data available to incorporate into the assessment; rather, it uses fault tree models in an elaborate but well-organized expert solicitation. Because we do not have yet have field data on gene-drive modified organisms, ecological risk assessment for gene drives will likely follow a similar approach as Hayes at al. The assessment is probabilistic and addresses uncertainty, and the authors used a Monte Carlo approach to address combinations of exposures and effects. However, the endpoints do not incorporate explicit stakeholder values and are essentially only measures of exposure.

Kuzma and Rawls (2016) have recently conducted an analysis that sets the stage for the application of ecological risk assessment to gene drives. The authors emphasized the importance of engagement with stakeholders and presented the multigenerational aspect of the release of a gene drive and its ramifications both for estimating effects and creating long-term management agreements.However, it is clear from the article's treatment of uncertainty that a great deal of specific information is missing that would have made this risk assessment more straightforward and useful for decision makers. The extensive information found in this document points to a variety of other information that may have proven useful to setting boundaries based on empirical data rather than expert elicitation. Although it addresses a non-driving modified organism and an accidental release scenario, Hayes et al., (2015) is the only risk assessment the committee could identify that follows the model put forth by Van den Brink et al., (2016).

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Appendix F

Biographical Sketches of Committee Members

From left to right: Angela Kolesnikova, Ann Kingiri, James Collins (co-chair), David Winickoff, Stephen Higgs, Gregory Kaebnick, Nicole Achee, Wayne Landis, Vicki Chandler, Lisa Taneyhill, Lynn Riddiford, Brandon Gaut, Joyce Tait, Joseph Travis, Elizabeth Heitman (co-chair), Frances Sharples, Audrey Thévenon, Paul Turner, Jason Delborne, Keegan Sawyer (Study Director).

Co-Chairs:

James P. Collins, PhD, is Virginia M. Ullman Professor of Natural History and the Environment in the School of Life Sciences at Arizona State University (ASU). Dr. Collins's research focuses on the role of host-pathogen interactions in species decline and extinction. His research group uses amphibians, along with viral and fungal pathogens, as models for studying factors that control population dynamics. Dr. Collins's also works on issues related to ecological ethics and the institutional and intellectual factors that have shaped the development of ecology as a discipline. From 1989 to 2002 he was Chair of ASU's Zoology, and then Biology Department, where he used interdisciplinary programs to foster innovation in research, education, and institutional change. He also was a founding director of ASU's Undergraduate Biology Enrichment Program, and served as co-director of ASU's Undergraduate Mentoring in Environmental Biology and Minority Access to Research Careers programs. Collins's expertise in population dynamics led him to serve as Director of the Population Biology and Physiological Ecology program at the National Science Foundation (NSF) from 1985 to 1986. He also served as NSF's Assistant Director responsible for Biological Sciences, one of seven NSF research directorates. He oversaw a science funding portfolio that spanned molecular biology to global change research, biological infrastructure, and biology

education. Dr. Collins is a Fellow of the American Association for the Advancement of Science (AAAS) and the Association for Women in Science (AWIS), and Past President of the American Institute of Biological Sciences (AIBS). He has been a member of numerous review panels for basic research and graduate training programs at NSF. He served as the Chairman of the US National Science and Technology Council's Committee on Science Subcommittee on Biotechnology (2005-2009) and the National Academy of Sciences Committee on Thinking Evolutionarily: Making Biology Education Make Sense (2011). Currently, Dr. Collins is Chair of the National Academy of Science's Board on Life Sciences.

Elizabeth Heitman, PhD, is Associate Professor of Medical Ethics in the Center for Biomedical Ethics and Society at Vanderbilt University Medical Center. Dr. Heitman's work focuses on cultural issues and international aspects of ethics in medicine, biomedical science, and public health. Her research examines international standards of research ethics, education in the responsible conduct of research, and trainees' awareness of professional and cultural norms. She is co-director of the research ethics education program "Formação Colaborativa na Ética em Pesquisa (Collaborative Research Ethics Education)", sponsored by the NIH Fogarty International Center, with colleagues from the Universidade Eduardo Mondlane in Maputo, Mozambique. Dr. Heitman previously directed a similar program with the Hospital Nacional de Niños in San José, Costa Rica and was PI of the National Science Foundation-funded study "Research Integrity in the Education of International Science Trainees." Dr. Heitman leads the research ethics activities of the Vanderbilt Institute for Clinical and Translational Research (VICTR), and coordinates VICTR's educational programs in the responsible conduct of research. She is a member of the National Academy of Science's (NAS) Board on Life Sciences, and its Standing Committee on Educational Institutes for Teaching Responsible Science. Through the NAS, Dr. Heitman has served as a faculty member in international faculty development projects on responsible science in the Middle East and North Africa, as well as Malaysia and Indonesia. She recently chaired the NAS Committee on the Elaboration of a National Curriculum in Bioethics and Responsible Conduct of Science for Algeria, advising the Algerian Ministry of Higher Education. Since 2009 she has been a member of the American Association for the Advancement of Science's (AAAS) Science Ethics Initiative with the China Association for Science and Technology, and has contributed to AAAS's work on biosafety/ biosecurity education since 2008. Dr. Heitman received her PhD in Religious Studies in 1988 from Rice University's joint program in biomedical ethics with the University of Texas – Houston Medical School.

Members:

Nicole L. Achee, PhD, is a Medical Entomologist (Research Associate Professor) within the Department of Biological Sciences and holds a joint Associate Professor appointment in the Eck Institute for Global Health at the University of Notre Dame. She has over 20 years of experience in vector behavior research related to the epidemiology and control of arthropod-borne diseases, including evaluation of vector ecology, habitat management and adult control strategies, disease risk mapping using GIS and remote sensing technologies, and evaluation of mosquito vector control products under both laboratory and field conditions. She has worked in the international settings of Belize, Indonesia, Mexico, Nepal, Peru, South Korea, Suriname, Tanzania and Thailand. Achee was the principal investigator of a research program funded by the Bill & Melinda Gates Foundation focused on the development of spatial repellents for use in combination push-pull systems to reduce human-vector contact for dengue prevention. She is currently a Principal Investigator for a multicenter intervention trial to generate evidence of the protective efficacy of spatial repellents for prevention of malaria and dengue human infections for use towards WHO recommendations. Achee is a Working Group member for the WHO Pesticide Evaluation Scheme (WHOPES), served as Chair of the American Committee of Medical Entomology (ACME) and is currently a Councilor of the American Society for Tropical Medicine and Hygiene (ASTMH), a member of the WHO Global Collaboration for the Development of Pesticides for Public Health partnership (GCDPP), a Vector Control Working Group representative of Roll Back Malaria and served as the lead scientist for the recent publication of the WHO Guidelines for Efficacy Testing of Spatial Repellents. Her latest efforts have

been dedicated to co-Directing the Belize Vector and Ecology Center (BVEC) in Belize to serve as a local platform of excellence for research, training and education in public health. Dr. Achee received a Ph.D. from the Uniformed Services University of the Health Sciences, a MSc. from Texas A&M University and a B.S. from Saint Louis University.

Vicki Chandler, PhD, (NAS) is Dean of the College of Natural Sciences at the Minerva Schools at the Keck Graduate Institute of Applied Life Sciences. Dr. Chandler has conducted critical research in the field of plant genetics for three decades and is recognized as one of the foremost geneticists in the world. In 2014, she was appointed to the National Science Board by President Barack Obama for a six-year term. Prior to Minerva, Chandler served as the Chief Program Officer for Science at the Gordon and Betty Moore Foundation. Prior to joining the Foundation, she was a Professor at the University of Oregon and the University of Arizona. She is passionate about helping students develop the skills they need to be successful in their future careers, part of which is directing them to be curious, lifelong learners. Dr. Chandler was a postdoctoral fellow at Stanford University, received a Ph.D. from the University of California San Francisco, and a B.A. from the University of California Berkeley. Dr. Chandler also served as President for the American Society of Plant Biologists in 2002 and the President of the Genetics Society of America in 2014.

Jason A. Delborne, PhD, is Associate Professor of Science, Policy, and Society in the Department of Forestry and Environmental Resources at North Carolina State University. Delborne joined NC State in August 2013 as part of the Chancellor's Faculty Excellence Program cluster in Genetic Engineering and Society. Delborne's research focuses on highly politicized scientific controversies, such as agricultural biotechnology, nanotechnology, biofuels, and climate change. Drawing upon the highly interdisciplinary field of Science, Technology, and Society (STS), he engages various qualitative research methodologies to ask questions about how policymakers and members of the public interface with controversial science. He also studies models for public engagement with science and technology, and the governance of emerging technologies. One of his current projects compares multiple pathways of development of genetically modified trees by exploring the extent to which responsible innovation is pursued and achieved. Delborne teaches and advises students affiliated with NC State's Genetic Engineering and Society Center and has published peer-reviewed articles in journals such as Social Studies of Science, Public Understanding of Science, and Science and Public Policy.

Brandon S. Gaut, PhD is Professor of Ecology and Environmental Biology at the University of California at Irvine (UCI). Dr. Gaut has been a faculty member at UCI since 1998. He served as Chair of the Department from 2006 to 2012 and Interim Dean of the School of Biological Sciences in 2013. Dr. Gaut's research focuses on the balance of forces that contribute to evolutionary change in plant populations, with particular emphasis on evolutionary genetics and comparative genomics of plant systems, including the genetics of domestication. Another dimension of his research is the evolution of transposable elements, sequences of DNA that move from one location in the genome to another, and how they contribute to genome differentiation and interspecific divergence. Dr. Gaut is the recipient of numerous honors, and investigator and teaching awards including UCI Professor of the Year, Outstanding Professor, and Biological Sciences Excellence and Teaching. He is an elected fellow of the American Association for the Advancement of Science, Senior Editor for Molecular Biology and Evology, and serves on the editorial board of Genome Biology and Evolution. Dr. Gaut also served as President for the Society of Molecular Biology and Evolution in 2014. Under the mentorship of Michael T. Clegg (member of the National Academy of Sciences), Dr. Gaut received his PhD in Plant Population Genetics from the University of California, Riverside.

Stephen Higgs, PhD, is the Virginia and Perry Peine Biosecurity Chair, Director of Biosecurity Research Institute (BRI), and Associate Vice President for Research at Kansas State University. The BRI is a secure biosafety level-3 and biosafety level-3 agriculture facility at Pat Roberts Hall. It enables studies on

diseases that impact the global food supply, including those affecting humans, livestock and plants as well as food-borne pathogens. Collaborative research, education and training is conducted at the BRI by faculty and staff from multiple departments, federal agencies and industry. Dr. Higgs is responsible for oversight, coordination and expansion of BRI's multidisciplinary research and education programs. He also serves as associate vice president for research, facilitating bio-preparedness research campus-wide. Dr. Higgs' research interests are mosquito-virus-vertebrate interactions, and is an expert in vector biology, arthropod-borne infectious diseases, immune modulation and vaccine evaluation. He is experienced in developing collaborative, multidisciplinary research projects and has organized training in biocontainment facilities for researchers from other universities and other countries. He has published more than 150 peer-reviewed papers and 16 book chapters, and has been a member of numerous national and international research program review panels. Higgs is the President of the American Society of Tropical Medicine and Hygiene (ASTMH), and is a fellow of both the ASTMH and the Royal Entomological Society. He also is editor-in-chief of the international journal Vector-Borne and Zoonotic Diseases, and an editorial board member of Health Security (formerly Biosecurity and Bioterrorism). Higgs earned a doctorate in parasitology from Reading University in the United Kingdom and a bachelor of science with honors in zoology from King's College in London. He was involved in training and research at the London School of Hygiene and Tropical Medicine and at the Institute of Virology and Environmental Microbiology, Oxford, in the United Kingdom before coming to the United States in 1991.

Gregory E. Kaebnick is a research scholar at The Hastings Center and editor of the Hastings Center Report. He is interested in questions about the values at stake in developing and using biotechnologies, and particularly in questions about the value given to nature and human nature. Dr. Kaebnick is the author of Humans in Nature: The World As We Find It and the World As We Create It (Oxford 2014), editor (with Thomas H. Murray) of Synthetic Biology and Morality: Artificial Life and the Bounds of Nature (MIT 2013), editor of The Ideal of Nature: Debates about Biotechnology and the Environment (Johns Hopkins 2011), and editor of Taking Sides: Clashing Views on Bioethical Issues. He participates in research projects at The Hastings Center on ethical issues in emerging biotechnologies. He is the principal investigator on a project funded by the National Science Foundation that explores the use of cost-benefit analysis and risk assessment for applications of synthetic biology. He served as a co-investigator on two research projects funded by the Alfred P. Sloan Foundation on ethical issues in synthetic biology and as principal investigator of a project funded by the National Endowment for the Humanities on appeals to nature in moral debates about biotechnology and the environment. He received his Ph.D. (1998) in philosophy from the University of Minnesota and his B.A. (1986) in religion from Swarthmore College.

Ann Kingiri, PhD, is a Senior Research Fellow at African Centre for Technology Studies (ACTS), a knowledge think tank based in Nairobi, Kenya. She is also a visiting researcher at the Development Policy and Practice (DPP) unit, Department of Engineering and Innovation, Open University, UK. Dr. Kingiri's technical expertise ranges across Science, Technology and Innovation (STI) policy analysis and advocacy; environmental policy analysis; biotechnology regulation, climate change; agriculture and food security; inclusive and sustainable development; gender research and analysis; and qualitative research methods. She is particularly interested in understanding these research areas from a Science Technology and Innovation (STI) perspective in relation to inclusive and sustainable development. She is currently pursuing policy-oriented research in agriculture and bioenergy, including climate change and gender as cross cutting themes. As a Senior Research Fellow at ACTS, Dr. Kingiri is responsible for the leadership of research to support the Science and Technology policy oriented capacity building, policy outreach and advocacy. She has been providing results oriented research and scientific leadership across the different programmes and projects being implemented by ACTS as well as STI mentorship. Before joining ACTS in 2011, she worked with the Ministry of Agriculture as an agricultural officer, with Kenya Plant Health Inspectorate Service (KEPHIS) as a phytosanitary and biosafety/biosecurity expert. While at KEPHIS, she was extensively involved in development of biotechnology and biosafety regulatory policies in Kenya. Dr. Kingiri has ample experience in networking and advocacy in a multicultural setting involving di-

verse development and policy actors in the public and private sector. Her previous involvement as a research fellow in the Research into Use (RIU) programme implemented in both Africa and Asia exposed her to the institutional and organisational orientation of agricultural entrepreneurship including the role of the private sector in stimulating innovation. Dr. Kingiri holds a PhD in Development Policy and Practice from Open University, UK. Additionally, she holds a Master's degree in Biosafety in Plant Biotechnology form Mache Polytechnic University, Ancona, Italy; an MSc degree in Plant Pathology from the University of Nairobi; and a BSc degree in Agriculture from the University of Nairobi.

Wayne Landis, PhD, is Professor and Director of the Institute of Environmental Toxicology at Western Washington University. Dr. Landis' current area of research is ecological risk assessment at large spatial and temporal scales. Dr. Landis' research contributions also include: creation of the Action at a Distance Hypothesis for landscape toxicology, the application of complex systems theory to risk assessment, and development of the Relative Risk Model for multiple stressor and regional-scale risk assessment and specialized methods for calculating risk due to invasive species and emergent diseases. He also has patents and papers on the use of enzymes and organisms for the degradation of chemical weapons. Dr. Landis has authored over 130 peer-reviewed publications and government technical reports, made over 300 scientific presentations, edited four books, and wrote the textbook, Introduction to Environmental Toxicology, now in its fourth edition. He has consulted for industry; non-governmental organizations as well as federal (U.S. and Canada), state, provincial, and local governments. Dr. Landis serves on the editorial boards of the journals Human and Ecological Risk Assessment and Integrated Environmental Assessment and Management, and is the ecological risk area editor for Risk Analysis. He is a member of the Society of Environmental Toxicology and Chemistry (SETAC) and served on the SETAC Board of Directors from 2000-2003. In 2007 he was named a Fellow of the Society for Risk Analysis. He was recently named to the Science Panel for the Puget Sound Partnership, a state agency that focuses on the restoration of Puget Sound. Dr. Landis received his PhD in Zoology (Indiana University), MA in Biology (Indiana University), and his BA in Biology (Wake Forest University).

Lynn Riddiford, PhD, (NAS) is a Senior Fellow at the Janelia Research Campus of the Howard Hughes Medical Institute and Professor of Biology Emeritus at the University of Washington. Her research focuses on the hormonal control of insect growth, molting, and metamorphosis, particularly the roles of ecdysone and juvenile hormone. She is also interested in the hormonal basis of metamorphic and reproductive behaviors. Dr. Riddiford pioneered in vitro approaches for studying the molecular mechanism of the major insect developmental hormones. Her basic studies on hormone action has aided in the development of hormone mimics for insect control. Dr. Riddiford is a member of the National Academy of Sciences, a Fellow of the American Academy of Arts and Sciences, AAAS, and the Entomological Society of America, and an Honorary Fellow of the Royal Entomological Society in England. She received the first Recognition Award in Insect Physiology, Biochemistry, and Toxicology from the Entomological Society of America in 1997, the G. J. Mendel Honorary Medal for Merit in the Biological Sciences from the Academy of Sciences of the Czech Republic in 1998, and the Vollum Award from Reed College in 2011. She was President of the American Society of Zoologists in 1991-92, the Councils of the International Congress of Entomology from 2000 to 2004, and the Federation of International Comparative Endocrinological Societies from 2001 to 2005. In addition, she has served on review and advisory panels for the National Science Foundation, the National Institutes of Health, the U.S. Department of Agriculture, the Scientific Advisory Board of the Whitney Marine Laboratory, and the Board of Directors of the Entomological Foundation, and the Governing Council of the International Center for Insect Physiology and Ecology, Nairobi, Kenya.

Joyce Tait, PhD, is Director of the Innogen Institute, and a professor at the University of Edinburgh. She has an interdisciplinary background in natural and social sciences, covering agrochemical, pharmaceutical and life science industry sectors, focusing on: strategic planning for innovation; governance, risk management and regulation; and stakeholder attitudes and influences. Relevant life science areas include syn-

thetic biology, genetic databases, GM crops, biofuels, pharmaceuticals, stem cell therapies and translational medicine. She is a Fellow of the Royal Society of Edinburgh and also of the Society for Risk Analysis. Current and recent appointments include: John Innes Centre Science and Impact Advisory Board; UK Department for Business Innovation and Skills (BIS) Synthetic Biology Leadership Council (Chair of Governance Subgroup); UK Department of Health Emerging Science and Bioethics Advisory Committee; Board of Directors, Roslin Foundation; Scottish Science Advisory Council; Scientific and Technical Council of the International Risk Governance Council, Geneva. Dr. Tait received her BSc from Glasgow University and her PhD from the University of Cambridge.

Lisa Taneyhill, PhD, is an Associate Professor in the Department of Animal and Avian Sciences at the University of Maryland, College Park. Dr. Taneyhill earned her Masters and Ph.D. degrees in Molecular Biology from Princeton University and completed postdoctoral work at the California Institute of Technology. To support her postdoctoral training, Dr. Taneyhill received a National Research Service Award (NRSA) from the NIH, and she was also one of the first recipients of the NIH K99/R00 Pathway to Independence Award. Dr. Taneyhill's lab explores how cellular junctions, akin to the molecular "velcro" that keeps cells together, are dismantled to generate migratory cell types and later reassembled to allow multiple cell types to interact to create new tissues and organs. This research is significant and will impact society by enhancing our understanding of the molecular mechanisms underlying the generation of migratory cells, a process co-opted during human diseases such as cancer, and the intercellular interactions required to create more complex structures in an embryo or adult organism. Dr. Taneyhill's research has advanced the field of developmental biology by describing the function, and dynamic modulation of, cellular junction components during embryonic development. Dr. Taneyhill has received funding from the NSF, NIH, and the American Cancer Society, as well as numerous accolades, including the College of Agriculture and Natural Resources Junior Faculty, Outstanding Faculty Advisor, and Outstanding Faculty Educator Awards. Dr. Taneyhill serves as a reviewer for numerous journals and on both NIH and NSF grant panels, and as a committee member for 18 Masters and Ph.D. students at the University of Maryland. She served as the principal organizer for the 2009 Mid-Atlantic Regional Society for Developmental Biology annual meeting and the 2015 Society for Craniofacial Genetics and Developmental Biology meeting, and is the author of 26 peer-reviewed publications, included three review articles and three book chapters.

Joseph Travis, PhD, is the Robert O. Lawton Distinguished Professor of Biological Science at Florida State University. He received his undergraduate degree from the University of Pennsylvania and his doctoral degree from Duke University. Travis joined the faculty in Biological Science at Florida State in 1980 and has served as Chair of the Biological Science Department (1991-1997), Director of the Program in Computational Science (2000-2005) and Dean of the College of Arts and Sciences (2005-2011). In his research, Travis works at the interface of ecology and evolutionary biology. The main goal of his research has been to understand why individuals from different populations of the same species often have very different features like the age at reproductive maturity or the rates of offspring production. In technical terms, Travis studies local adaptations and how multiple ecological forces combine to generate different pressures of natural selection in different locations. His current research is focused on the evolution of life history and ecological interactions in populations of Trinidadian guppies, divergence in the responses of the molecular stress network in populations of least killifish, and indirect genetic effects among genotypes of male mosquitofish. Travis has taught a diversity of classes at Florida State University and, in many summers, at the Mountain Lake Biological Station at the University of Virginia. These have included Herpetology, Vertebrate Biology, Field Ecology, Quantitative Methods, Experimental Biology, Behavioral Ecology, Population Ecology, and Evolution. He has supervised 8 completed M.S. theses and 18 completed doctoral dissertations. Travis has served on the editorial boards of Journal of Evolutionary Biology, Oecologia, Annual Review of Ecology and Systematics, and The American Naturalist. He served as editor of The American Naturalist from 1998 to 2002 and as Vice-President (1994) and President (2005) of the American Society of Naturalists. He served as President of the American Institute of Biological Sci-

ences in 2010 and is serving again from 2013 through 2016. He has served on advisory panels for the National Science Foundation, the National Marine Fisheries Service, and the National Center for Ecological Analysis and Synthesis. In 1991, he was elected a Fellow of the American Association for the Advancement of Science and in 2011 received the E. O. Wilson Naturalist Award from the American Society of Naturalists. In 2015 he was elected as a Fellow of The American Academy of Arts and Sciences.

Paul Turner, PhD, is currently Professor and Departmental Chair of Ecology and Evolutionary Biology at Yale University, and a faculty member in the Microbiology Graduate Program at Yale School of Medicine. Dr. Turner was elected Councilor for Division R (Evolutionary and Genomic Microbiology) of the American Society for Microbiology, and Councilor for the American Genetic Association, and currently serves on the Biological Sciences Advisory Committee of the National Science Foundation. Dr. Turner was elected chair of several international meetings, including the 2013 Gordon Research Conference on Microbial Population Biology, and the 2018 Jacques Monod Conference on Viral Emergence. He has authored nearly 100 scholarly journal articles, reviews and book chapters, and has served as Associate Editor for journals such as Evolution, and Evolution, Medicine and Public Health. Dr. Turner also served on the National Academy of Sciences committee on Biological Confinement of Genetically Engineered Organisms. Dr. Turner's work involves basic research in microbial evolution and the evolution of infectious diseases, often harnessing laboratory populations of viruses as model systems to study mechanisms of evolutionary change. He also conducts applied research on novel approaches to treat infectious diseases of humans and other organisms. Dr. Turner heads a research group with diverse interests; current members are using microbes to address questions relating to the evolution of genetic exchange (sex), host-parasite interactions, pathogen emergence, virus biogeography, the ecology and evolution of infectious disease, and development of novel antimicrobials. His research program is highly inter-disciplinary, employing techniques from microbiology, population genetics, genomics, molecular biology and mathematical modeling. Dr. Turner's lab website is: http://turnerlab.yale.edu/. Dr. Turnter received his Ph.D. in 1995 from the Center for Microbial Ecology, at Michigan State University and completed postdoctoral work at the National Institutes of Health, University of Valencia in Spain, and University of Maryland, College Park.

David E. Winickoff, JD, is Associate Professor of Bioethics at University of California, Berkeley in the College of Natural Resources where has been located since 2004. Currently, he is serving as a Senior Policy Analyst and Secretary of the Working Party on Bio-, Nano- and Converging Technology at the Organization for Economic Cooperation and Development (OECD) in Paris. Broadly speaking, his work attempts to help solve difficult ethical, legal and social problems at the interface of science, technology and society, especially related to the environment and human health. He draws questions and methods from the fields of science and technology studies (STS), ethics, and the law. In particular, he analyzes the role of science and experts within environmental law and governance across local and global scales; he studies the practices and regulation of emerging technologies like genetic modification, human genomics, and geoengineering; and how the ethics and politics of manipulating nature and natural systems using advanced life science. He has over forty publications in academic journals and other outlets. His articles have appeared in Science, New England Journal of Medicine, Nature Climate Change and the Yale Journal of International Law, among others. Winickoff served as a Working Group member on a Royal Academy project on geoengineering, and sits on a number of bioethics advisory boards around the United States. At Berkeley, he directs the Program in Science and Technology Studies. He holds degrees from Yale, Cambridge, and Harvard Law School and was a fellow for two years at the Harvard Kennedy School.