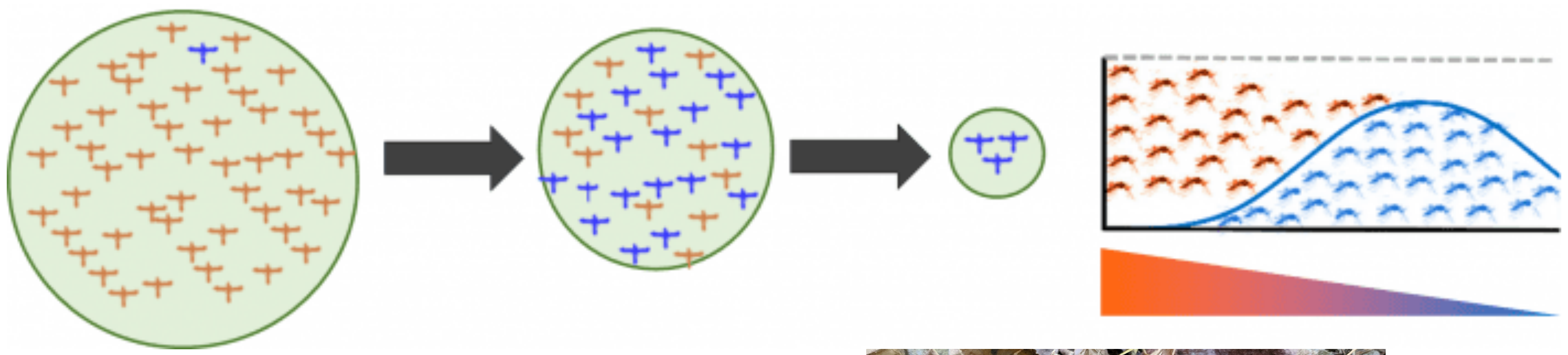
# Gene Drive Risk Assessment

## Developing Approaches

Hector Quemada ISAAA Webinar June 16, 2022

# Gene Drive Research

Range of applications



- Disease control
  - Mosquitoes (malaria)
  - Rodents (ticks → Lyme disease)
- Conservation/invasive species elimination
  - Rodents (bird predation)
  - Cane toads
  - Fish
  - Golden mussel
- Agriculture (pest elimination)
  - Rodents
  - Insects

# Few Projects Have Conducted Biosafety-Related Work

- Early stages: too soon to do project-specific work for most projects
- Some preliminary work has been done
  - Problem formulation workshops
    - FNIH
    - AUDA/NEPAD
    - EFSA

Roberts et al. AJTMH 2017

PROBLEM FORMULATION FOR GENE DRIVE IN MOSQUITO

**Perspective Piece**

**Results from the Workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes"**

Andrew Roberts,[1]* Paulo Paes de Andrade,[2] Fredros Okumu,[3] Hector Quemada,[4] Moussa Savadogo,[5] Jerome Amir Singh,[6,7] and Stephanie James[8]

Teem et al. Malar J (2019) 18:347
https://doi.org/10.1186/s12936-019-2978-5

Malaria Journal

RESEARCH — Open Access

Problem formulation for gene drive mosquitoes designed to reduce malaria transmission in Africa: results from four regional consultations 2016–2018

John L. Teem[1], Aggrey Ambali[2], Barbara Glover[2], Jeremy Ouedraogo[3], Diran Makinde[2] and Andrew Roberts[1]*

  - Problem formulation for one potential product

Connolly et al. Malar J (2021) 20:170
https://doi.org/10.1186/s12936-021-03674-6

Malaria Journal

RESEARCH — Open Access

Systematic identification of plausible pathways to potential harm via problem formulation for investigational releases of a population suppression gene drive to control the human malaria vector *Anopheles gambiae* in West Africa

John B. Connolly[1]*, John D. Mumford[2], Silke Fuchs[1], Geoff Turner[1], Camilla Beech[3], Ace R. North[4] and Austin Burt[1]

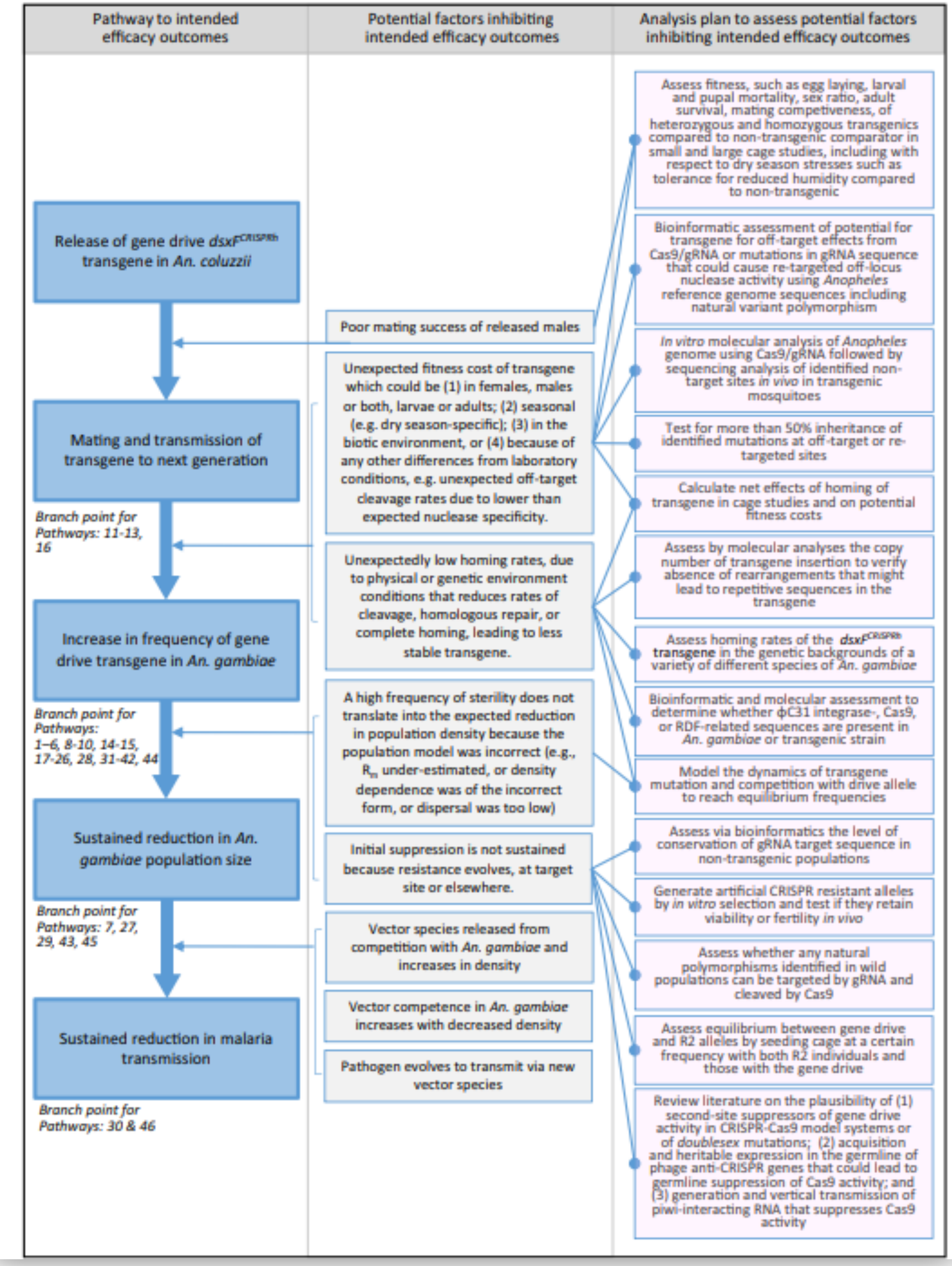
efsa European Food Safety Authority

EVENT REPORT

APPROVED: 9 March 2020
doi:10.2903/sp.efsa.2020.EN-1819

**Stakeholder workshop "Problem formulation for the environmental risk assessment of gene drive modified insects" (15 May 2019, Brussels)**

European Food Safety Authority (EFSA),
Yann Devos, Barbara Gallani and Leslie G Firbank


Pathway to intended efficacy outcomes
Potential factors inhibiting intended efficacy outcomes
Analysis plan to assess potential factors inhibiting intended efficacy outcomes
Release of gene drive dsxF^CRISPRh transgene in An. coluzzii
Mating and transmission of transgene to next generation
Branch point for Pathways: 11-13, 16
Increase in frequency of gene drive transgene in An. gambiae
Branch point for Pathways: 1–6, 8-10, 14-15, 17-26, 28, 31-42, 44
Sustained reduction in An. gambiae population size
Branch point for Pathways: 7, 27, 29, 43, 45
Sustained reduction in malaria transmission
Branch point for Pathways: 30 & 46
Poor mating success of released males
Unexpected fitness cost of transgene which could be (1) in females, males or both, larvae or adults; (2) seasonal (e.g. dry season-specific); (3) in the biotic environment, or (4) because of any other differences from laboratory conditions, e.g. unexpected off-target cleavage rates due to lower than expected nuclease specificity.
Unexpectedly low homing rates, due to physical or genetic environment conditions that reduces rates of cleavage, homologous repair, or complete homing, leading to less stable transgene.
A high frequency of sterility does not translate into the expected reduction in population density because the population model was incorrect (e.g., Rm under-estimated, or density dependence was of the incorrect form, or dispersal was too low)
Initial suppression is not sustained because resistance evolves, at target site or elsewhere.
Vector species released from competition with An. gambiae and increases in density
Vector competence in An. gambiae increases with decreased density
Pathogen evolves to transmit via new vector species
Assess fitness, such as egg laying, larval and pupal mortality, sex ratio, adult survival, mating competiveness, of heterozygous and homozygous transgenics compared to non-transgenic comparator in small and large cage studies, including with respect to dry season stresses such as tolerance for reduced humidity compared to non-transgenic
Bioinformatic assessment of potential for transgene for off-target effects from Cas9/gRNA or mutations in gRNA sequence that could cause re-targeted off-locus nuclease activity using Anopheles reference genome sequences including natural variant polymorphism
In vitro molecular analysis of Anopheles genome using Cas9/gRNA followed by sequencing analysis of identified non-target sites in vivo in transgenic mosquitoes
Test for more than 50% inheritance of identified mutations at off-target or re-targeted sites
Calculate net effects of homing of transgene in cage studies and on potential fitness costs
Assess by molecular analyses the copy number of transgene insertion to verify absence of rearrangements that might lead to repetitive sequences in the transgene
Assess homing rates of the dsxF^CRISPRh transgene in the genetic backgrounds of a variety of different species of An. gambiae
Bioinformatic and molecular assessment to determine whether ϕC31 integrase-, Cas9, or RDF-related sequences are present in An. gambiae or transgenic strain
Model the dynamics of transgene mutation and competition with drive allele to reach equilibrium frequencies
Assess via bioinformatics the level of conservation of gRNA target sequence in non-transgenic populations
Generate artificial CRISPR resistant alleles by in vitro selection and test if they retain viability or fertility in vivo
Assess whether any natural polymorphisms identified in wild populations can be targeted by gRNA and cleaved by Cas9
Assess equilibrium between gene drive and R2 alleles by seeding cage at a certain frequency with both R2 individuals and those with the gene drive
Review literature on the plausibility of (1) second-site suppressors of gene drive activity in CRISPR-Cas9 model systems or of doublesex mutations; (2) acquisition and heritable expression in the germline of phage anti-CRISPR genes that could lead to germline suppression of Cas9 activity; and (3) generation and vertical transmission of piwi-interacting RNA that suppresses Cas9 activity

# Risk Assessments

- Wolbachia (Murray et al. 2016)
- Transgenic Mosquitoes

  CSIRO: Target Malaria

  Brown et al. (2022)
- Hypothetical mouse

  Brown et al. (2022)

frontiers in Public Health

ORIGINAL RESEARCH
published: 22 March 2016
doi: 10.3389/fpubh.2016.00043

CrossMark

**Risk Associated with the Release of *Wolbachia*-Infected *Aedes aegypti* Mosquitoes into the Environment in an Effort to Control Dengue**

*Justine V. Murray[1*], Cassie C. Jansen[1,2] and Paul De Barro[1]*

*[1]CSIRO, Brisbane, QLD, Australia, [2]Metro North Public Health Unit, Queensland Health, Brisbane, QLD, Australia*

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**Risk Assessment for Controlling Mosquito Vectors with Engineered Nucleases: Sterile Male Construct**
**Final report**

Keith R. Hayes, Simon Barry, Nigel Beebe, Jeffrey M. Dambacher, Paul De Barro, Scott Ferson, Jessica Ford, Scott Foster, Anders Gonçalves da Silva, Geoffrey R. Hosack, David Peel and Ronald Thresher
September 4, 2015

CSIRO HEALTH AND BIOSECURITY
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**Risk Assessment for Controlling Mosquito Vectors with Engineered Nucleases: Controlled field release for Sterile Male Construct**
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May 2, 2018

DOI: 10.1111/risa.13948

ORIGINAL ARTICLE

Journal of Risk Analysis

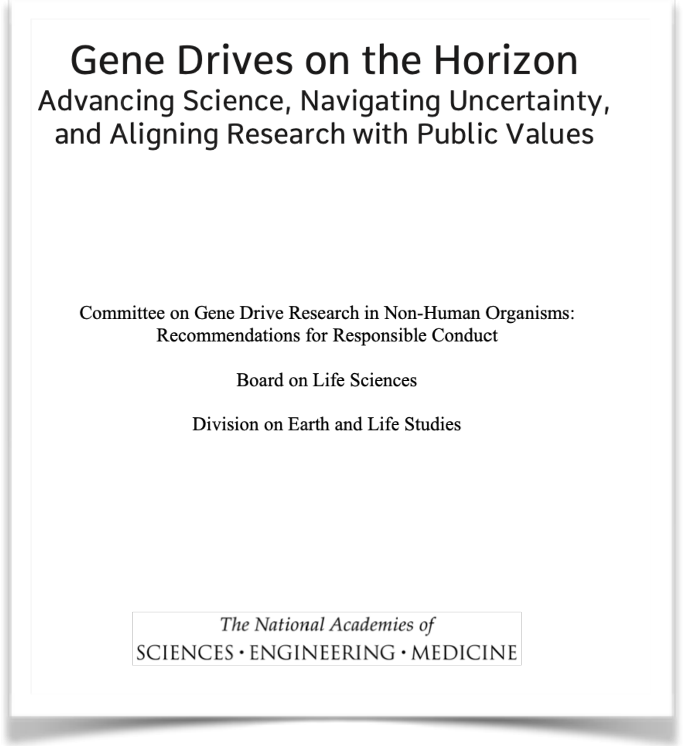
**Bayesian network-based risk assessment of synthetic biology: Simulating CRISPR-Cas9 gene drive dynamics in invasive rodent management**

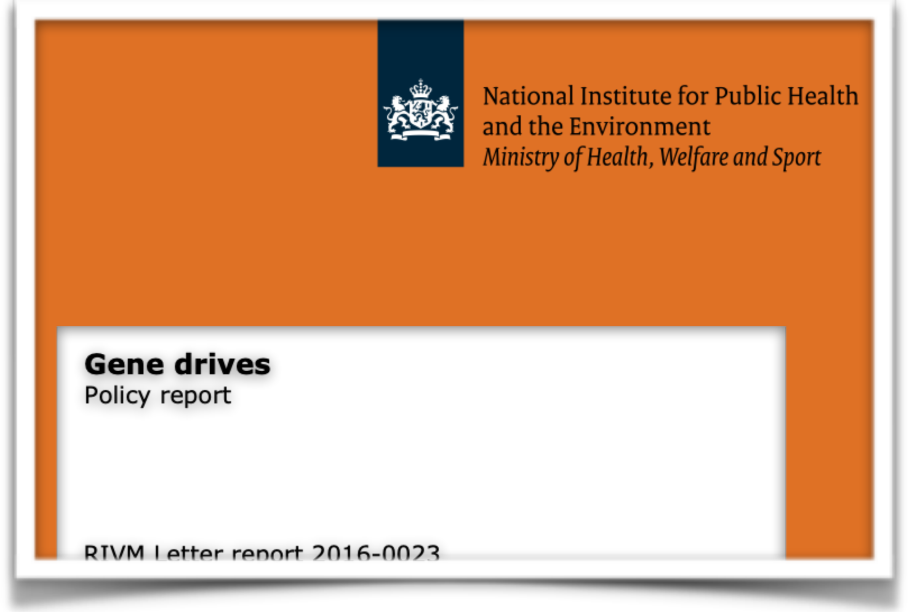
Ethan A. Brown | Steven R. Eikenbary | Wayne G. Landis

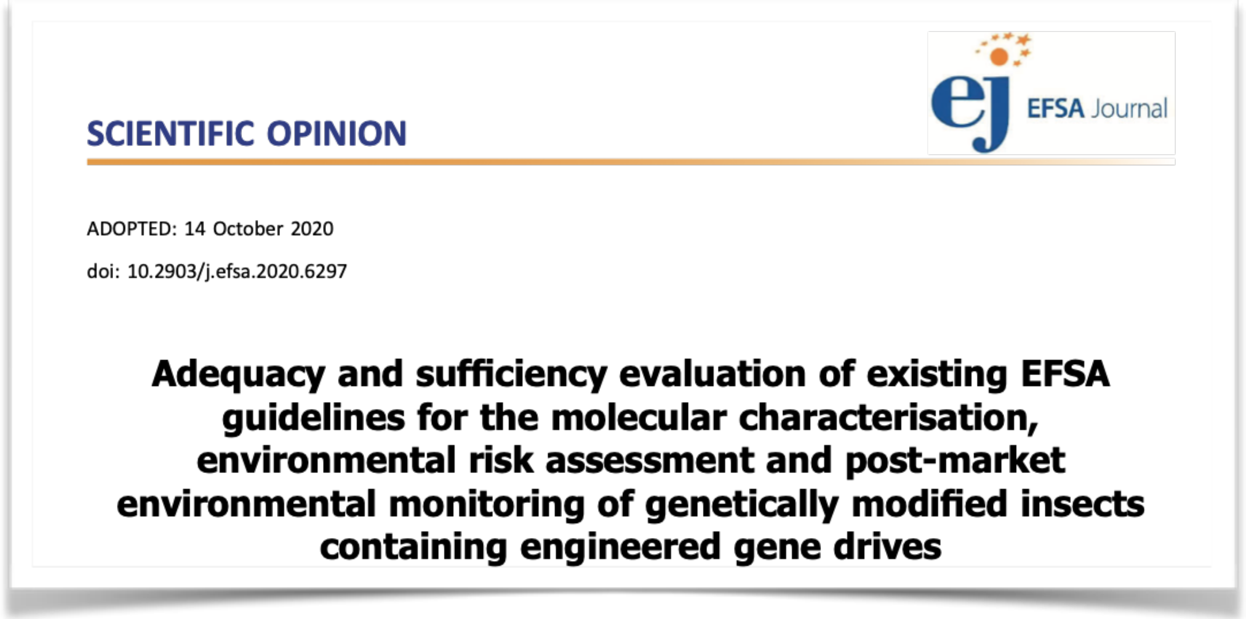
# Scientific Opinions

Most opinions have not indicated that new risk assessment methodologies are needed.

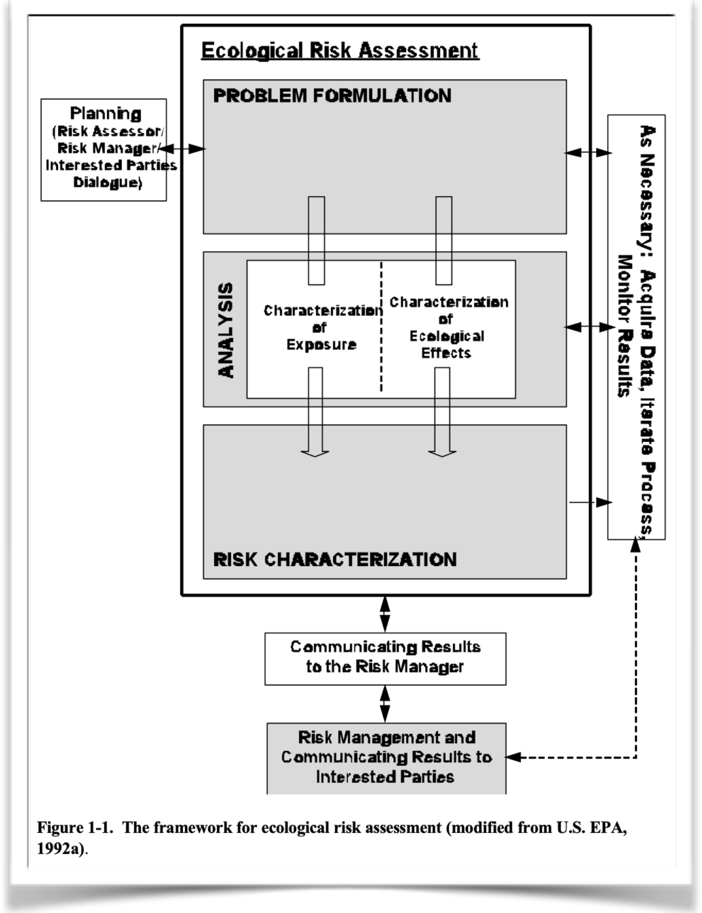
- US National Academy of Science, Engineering and Medicine
- Australian Academy of Science
- RIVM (Netherlands)
- European Food Safety Authority
- NExTRAC

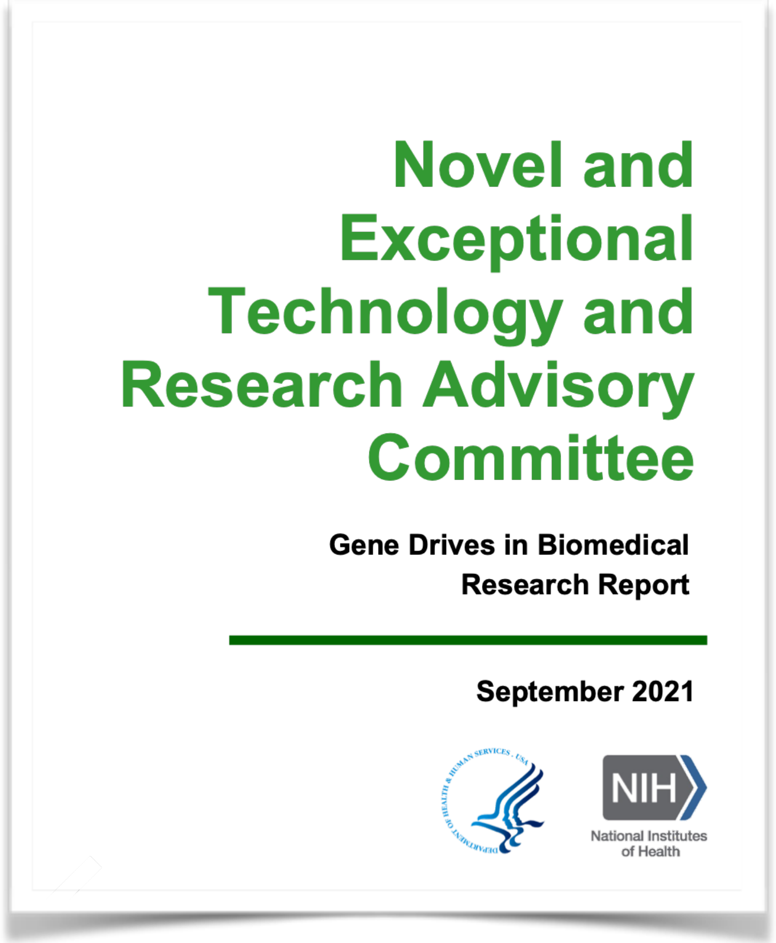






# Challenges For Risk Assessment

## EFSA

- Receiving environments
- Comparators
- Non-GM surrogates
- Experimental design and statistics
- Long-term effects
- Modelling
- Persistence and invasiveness, including vertical gene flow
- HGT
- Pathogens, infections and diseases
- Interactions with target organisms

## NExTRAC

- Balancing potential benefits/harms
- Comparing with existing interventions
- Dealing with ecological and evolutionary complexity
- Considering potential social and ethical benefits/harms
- Modeling with limited data
- Detecting rare events
- Identifying endpoints with stakeholder and community input
- Dealing with social and cultural complexity
- Managing uncertainty

# Other Disciplines

Risk assessments of organisms with similar characteristics can be used to supplement

- Classical biocontrol
- Invasive species
- Wolbachia

Biological Control 52 (2010) 245–254

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

ELSEVIER

Review

Progress in risk assessment for classical biological control

B.I.P. Barratt[a,*], F.G. Howarth[b], T.M. Withers[c], J.M. Ke

CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2011 **6**, No. 042

**Review**

**Assessing safety of biological control introductions**

**Barbara I.P. Barratt***

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***Correspondence:** Email: barbara.barratt@agresearch.co.nz

frontiers in Public Health

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Risk Analysis

AN INTERNATIONAL JOURNAL

An Official Publication of the Society for Risk Analysis

Risk Assessment for Invasive Species

Mark C. Andersen, Heather Adams, Bruce Hope, Mark Powell

# Areas of Concern Remain The Same

Snow et al. 2005

TABLE 2. Major environmental concerns regarding transgenic organisms.

| Process | Potential ecological consequences |
|---|---|
| Transgenic organisms persist without cultivation | Transgenic organisms that are able to spread and maintain self-sustaining populations could disrupt biotic communities and ecosystems, leading to a loss of biological diversity. |
| Transgenic organisms interbreed with related taxa | Incorporation of transgenes could result in greater invasiveness or loss of biodiversity, depending upon the amount of gene flow from generation to generation and the transgenic trait(s). |
| Horizontal gene flow | The transfer of genes through nonsexual means is common in some microbes but rare in plants and animals. Ecological consequences would depend on amount of gene flow and the transgenic trait(s). |
| Changes in viral disease | In transgenic virus-resistant organisms, recombination between viral transgenes and invading viruses could lead to increased virulence of a disease and undesirable effects on wild hosts in natural habitats. |
| Nontarget and indirect effects | Loss of biodiversity, including species of conservation concern, may occur, as well as altered community or ecosystem function, including reduced biological pest control, reduced pollination, altered soil carbon and nitrogen cycling, and secondary pest outbreaks. |
| Evolution of resistance | Resistance to pesticides (including pesticide-producing plants) can lead to greater reliance on chemicals and other pest control methods that are damaging to the environment, including unregistered pesticides under emergency exemptions. This applies to insects, weeds, and other pests. |

*Note:* Note that few types of transgenic organisms have been released into the environment, and therefore few of the potential ecological consequences listed have been documented to date (see *Ecological effects of GEOs* for details).

Snow 2019

***Table 1. Possible environmental risks of releasing genetically engineered (GE) vertebrates into natural habitats (adapted from Snow et al. 2005), with examples of hypothetical, worst-case scenarios for the current case study involving GE Lyme-resistant white-footed mice.***

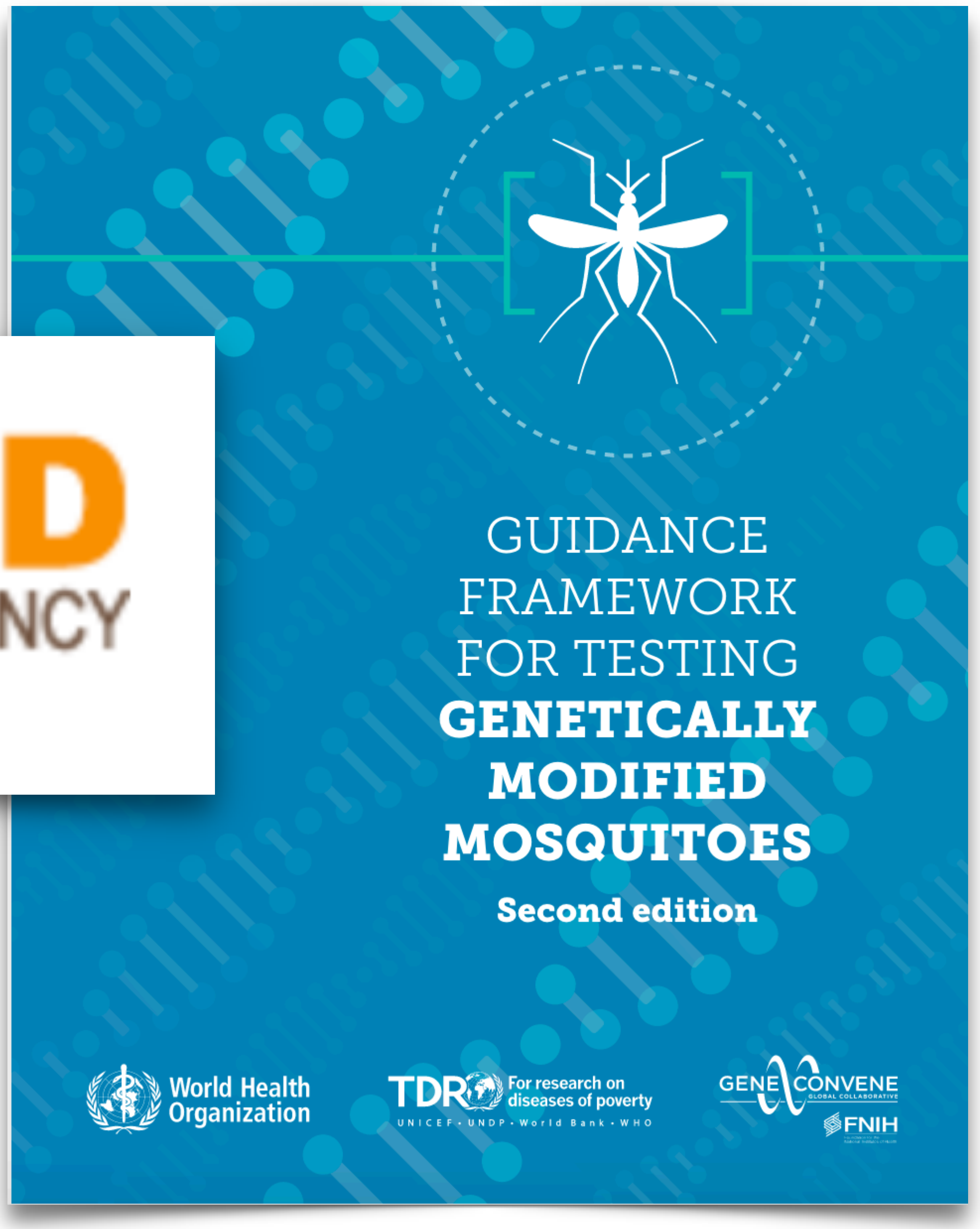
| Type of risk | Hypothetical scenario |
|---|---|
| Exacerbating effects of existing pests or pathogens. | ***Scenario 1:*** *Competitive release of more harmful tick-borne pathogens that may currently be suppressed by the frequent presence of Lyme spirochetes in white-footed mice.*<br>***Scenario 2:*** *Increased unwanted contact between humans and white-footed mice during any massive, pulsed introductions of tens to hundreds of thousands of GE mice.* |
| Facilitating the introduction and establishment of new pests or pathogens. | *No proposed scenario.* |
| Loss of genetic diversity within species. | ***Scenario 1:*** *Genetic bottlenecks that could occur during initial selection, lab-rearing procedures, and field releases of GE white-footed mice, perhaps leading to inbreeding depression, the loss of subspecies, or the loss of adaptation to local environments.* |
| Harm to other species, in some cases leading to a loss of species diversity. | ***Scenario 1:*** *Fitness costs or benefits associated with a novel GE trait in white-footed mice, resulting in altered abundance or population fluctuations, with unwanted cascading effects on other species.*<br>***Scenario 2:*** *Altered foraging behavior of GE Lyme-resistant white-footed mice, such as preying on eggs of ground-nesting birds to a greater extent when white-footed mice are not infected by Lyme spirochetes (Ostfeld et al. 2018b).* |
| Other unwanted disruption of biotic communities, including disruption of ecosystem services. | *No proposed scenario.* |
| Noncompliance with legal or regulatory requirements, or with ethical standards for research and deployment of GE animals. | ***Scenario 1:*** *Unintended dispersal and establishment of GE Lyme-resistant white-footed mice on the mainland or on other islands where regulatory approvals, environmental risk assessments, or public engagement are lacking. Long distance dispersal could occur via swimming or when white-footed mice become stowaways in boxes, gear, firewood, and other items that are transported by people (e.g., Scheppe 1965).* |

Note: See the text for details. The environmental benefits of releasing GE vertebrates (e.g., efforts to preserve endangered species) are not considered in the present article, nor are cases that involve gene drive systems.

# Guidance

- Generic
  - Cartagena Protocol Annex III (additional guidance to be written on gene drives)
  - WHO 2018 guidance
  - Relevant guidance for organisms with similar characteristics (Wolbachia)
- Gene Drive Specific
  - Contained use (Australia, Netherlands, ACME)
  - NEPAD West Africa Integrated Vector Management
  - Cartagena Protocol AHTEG


AUDA-NEPAD
AFRICAN UNION DEVELOPMENT AGENCY




Vector-Borne and Zoonotic Diseases > Vol. 22, No. 1 > Reviews    Open Access

**Containment Practices for Arthropods Modified with Engineered Transgenes Capable of Gene Drive Addendum 1 to the Arthropod Containment Guidelines, Version 3.2**

Enhanced ACL2

# Summary

- Most projects are still too early to do case-specific research on gene drive events
- Basic risk assessment approaches used with previous GE organisms are appropriate
    - Additional challenges need to be addressed
    - Borrow from risk assessments in other risk assessment disciplines
        - Handling uncertainty
        - Modeling
    - Borrow from risk assessments of other organisms
        - Classical biological control
        - Invasive species
        - Non-synthetic drives
- Value of additional guidance yet to be determined