April 2024 Changes to the NIH Guidelines

Gene Drive Modified Organisms

NIH has amended the NIH Guidelines to ensure the continued responsible research involving Gene Drive Modified Organisms (GDMOs) in contained research settings. The changes take effect at the end of September 2024.

Specifically, the NIH Guidelines will be amended to:

1. Clarify minimum containment requirements for research involving Gene Drive Modified Organisms (GDMOs);
2. Provide considerations for risk assessment;
3. Define additional institutional responsibilities for IBCs and BSOs.

In addition to the amendments related to contained research involving GDMOs, the NIH Guidelines will also be amended to:

4.Rreplace the term ‘‘helper viruses’’ with the broader term ‘‘helper systems’’;

‘‘The potential for reversion or generation of replication competent virus should be considered when generating or using defective viruses or vectors in the presence of helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems).’’

And

5.. Reclassify West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) as risk group 2 agents for consistency with containment guidance provided in the BMBL.

Gene Drive Modified Organisms (GDMO’s)

Definition of a Gene Drive:
Section I-E-7. “Gene drive” is defined as a technology whereby a particular heritable element biases inheritance in its favor, resulting in the heritable element becoming more prevalent than predicted by Mendelian laws of inheritance in a population over successive generations.

Field Release or field work with GDMO’s not allowed:
NIH does not currently support field release of GDMOs and the NIH Guidelines pertain to contained research. All research involving GDMOs must be conducted within a

research, plant or animal research laboratory after authorization from the Yale Biological Safety Committee.

GDMO Research Must be Registered with and Approved by the Yale Biological Safety Committee

GDMO research is NOT EXEMPTED from the NIH GUIDELINES:
Research subject to the NIH Guidelines, including research with GDMOs, requires review and approval by an IBC that is registered with the NIH Office of Science Policy prior to initiation.

The following categories are not exempt from the NIH Guidelines:

(i) experiments described in Section III-B, which require NIH OSP and Institutional Biosafety Committee approval before initiation;

(ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval;

(iii) large-scale experiments (e.g., more than 10 liters of culture),

(iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates), and

(v) experiments involving gene drive modified organisms (Section III-D-8).

This means that any GDMO research in E. coli K12, saccharomyces cerevisiae, B. subtilis, purchased transgenic rodents, or other exempt research must be registered and approved by the Yale Biological Safety Committee prior to initiation.

GDMO Research Requires a Minimum of BSL-2 Containment:

Research involving GDMOs must be conducted at a minimum containment level of BSL-2, BL2-N (Animal BSL-2), or BL2-P (Plant BSL-2). Based on the risk assessment of a specific research protocol, the IBC may require enhancements or a higher level of containment. Review and approval from NIH OSP are required to lower containment below the minimum specified in the NIH Guidelines.

> **Section III-D-8.** Experiments Involving Gene Drive Modified Organisms
> Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of Biosafety Level (BL) 2, BL2-N (Animals) or BL2-P (plant) containment.

When the institution conducts research involving GDMOs, the institution must ensure that the IBC has adequate expertise (e.g., specific species containment, ecological or environmental risk assessment) using ad hoc consultants if necessary.
(The Yale Biological Safety Committee has added consultants to our Committee to assist with the ecological risk assessment of GDMO in the event of an accidental release from the laboratory).

In addition, when such research is being conducted, a Biological Safety Officer (BSO) shall be appointed to the IBC. Yale University's Biosafety Officer is a representative of the Yale Biological Safety Committee.

Research involving gene drive modified organisms may require risk assessments that incorporate a broader scope of considerations because of greater uncertainty of the technology and potential uncertainty of the impact of the newly modified organism. Specific attention must be paid to risks of an unintended release from the laboratory and the potential impact on humans, other populations of organisms, and the environment.

Considerations for conducting risk assessments for research involving gene drive modified organisms might include:

1) The specific types of manipulations based on:

   a) Function or intended function of the genetic/gene drive construct (i.e., a designed or engineered assembly of sequences);

   b) Source of the genetic material (e.g., sequences of transgenes) in the construct;

   c) The modifications to the construct;

   d) Whether it is possible to predict the consequences of a construct, including the recognition of an unintended gene drive (i.e., construct not specifically designed as a gene drive but nonetheless having properties of a gene drive) and the possible consequences of escape into the environment;

   e) The potential ability of the gene drive to spread or persist in local populations;

2) Options for approaches to risk mitigation for specific types of risks in experiments or when dealing with a high degree of uncertainty about risks;

3) Considerations for implementing more stringent containment measures until biosafety data are accrued to support lowering containment.

The conduct of risk assessments for research involving GDMOs presents challenges in addition to those associated with other genetically modified organisms (GMOs) or vectors because the preferentially inherited traits of GDMOs spread and persist in the environment, are intended to modify natural populations, and may have associated impacts on the environment and society.

The potentially broad and long-lasting impacts of the use of this technology on humans, other populations of organisms, and the environment are not seen with research involving clinical research participant cohorts or even with other GMOs not designed to survive outside of laboratory containment. As such, research involving GDMOs requires risk assessments that incorporate a broader scope of issues because of the greater uncertainty in terms of risks in the event of an unintended release from the laboratory. The Yale Biological Safety Committee has updated its gene drive modified organism research questions to collect the information detailed below.

Risk assessments for research involving GDMOs:
Here are the questions you must answer for the Committee in order for the review of your Gene Drive Modified Organisms research.

Does your research involving recombinant or synthetic nucleic acid molecules involve the generation of a gene drive in humans, animals, plants, other organisms, bacteria, archaea, cells or viruses?

If no, could your research introduce an unintended gene drive?

If yes to either of the above, please identify the following:

GDMO Questions:

Host organism or cells that will be modified to include a gene drive:

Vector(s) utilized in GDMO research:

Inserted nucleic acids:

What is the intended function of the genetic/gene drive construct?

What is the source of the genetic material in the construct?

Describe the modifications to the construct?

What are the potential consequences of the GDMO construct created to humans, organisms, or the environment?

What are the consequences of the GDMO if it was released from the laboratory and into the environment?

Does the gene drive modified organism have the potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems?

Is the targeted gene present in non-laboratory populations?

Is the target gene essential to survival and reproduction?

Can the gene drive be transferred to non-target species?

What is the speed of reproduction of the gene drive modified organisms?

What is the likelihood that resistance to the transgene will be selected for if introduced into a natural population?

Is the gene drive technology designed to be self-limiting (i.e., have limited ability to spread outside of a given area – spatially and/or temporally)?

What is the estimated number of organisms that would need to be released for the trait to spread throughout a population?

What is the potential for ecological consequences if organisms spread or persist and how any consequences can be evaluated in the event of an inadvertent release?

Are there any options to mitigate risks for the gene drive in the event of a release?

What additional containment measures have been implemented for this gene drive research?

Sections of the April 2024 NIH Guidelines Impacted by Adding GDMOs

Physical Containment
Research involving GDMOs shall be conducted at a minimum of Biosafety Level 2 (BL2), BL2-N (certain animals) or BL2-P (plant) containment. Based on the risk assessment of a specific research protocol, the IBC may require enhancements or a higher level of containment. Review and approval from NIH OSP are required to lower containment below the minimum specified in the NIH Guidelines.

Experiments involving gene drive modified animals or experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms, except for viruses that are only vertically transmitted, may not be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required (see Section III-D-8).

> **Caution** - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms (e.g., a gene drive; refer to Section III-D-8) or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Experiments involving gene drive modified animals generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of BL2 or BL2-N (see Section III-D-8).

Section III-D-4-c-(3). Experiments involving the generation or use of gene drive modified animals require a minimum of BL2 containment and are covered under III-D-8, Experiments Involving Gene Drive Modified Organisms.

III-D-5-e. If experiments involving whole plants are not described in Section III-D-5 and do not fall under Sections III-A, III-B, III-D or III-F, they are included in Section III-E. Experiments involving the generation or use of gene drive modified organisms require a minimum of BL2 containment and are described under Section III-D-8, Experiments Involving Gene Drive Modified Organisms.

Section III-D-8. Experiments Involving Gene Drive Modified Organisms
Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of Biosafety Level (BL) 2, BL2-N (Animals) or BL2-P (plant) containment.

Section III-E-3. Experiments Involving Transgenic Rodents
This section covers experiments involving the generation or use of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under **Section III-D-4**, Experiments Involving Whole Animals or Section III-D-8, Experiments Involving Gene Drive Modified Organisms.

Section IV-B-1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant or synthetic nucleic acid molecule research at Biosafety Level (BL) 3 or BL4, (ii) engages in large-scale (greater than 10 liters) research or (iii) conducts any research involving gene drive modified organisms, which all must be conducted at BL2 or higher containment. The Biological Safety Officer carries out the duties specified in Section IV-B-3.

Membership of IBC:
When the institution conducts research involving gene drive modified organisms the institution must ensure that the Institutional Biosafety Committee has adequate expertise (e.g., specific species containment, ecological or environmental risk assessment) using ad hoc consultants if necessary. When the institution conducts recombinant or synthetic nucleic acid molecule research at BL3, BL4, or Large Scale (greater than 10 liters) or research involving gene drive modified organisms, a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, Biological Safety Officer). When the institution conducts research with gene drive modified organisms, the impact on ecosystems should be assessed by the Institutional Biosafety Committee (see Section V-N, Footnotes and References of Sections I-IV). When the institution participates in or sponsors recombinant or synthetic nucleic acid molecule research involving human research participants, the institution must ensure that the Institutional Biosafety Committee has adequate expertise and training (using ad hoc consultants if necessary). Institutional Biosafety Committee approval must be obtained from the clinical trial site.

Section IV-B-3-c. The institution shall appoint a Biological Safety Officer if it engages in recombinant or synthetic nucleic acid molecule research that involves gene drive modified organisms. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

Section V-N Determination of whether a gene drive modified organism has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of gene drive technology, and of the environment, and ecosystems in the geographic area of the research.

Appendix C-III-A. Exceptions
The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-B, which require NIH OSP and Institutional Biosafety Committee approval before initiation; (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, and Sections V-G and V-L, Footnotes and References of Sections I through IV) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety

Committee review and approval; (iii) large-scale experiments (e.g., more than 10 liters of culture), (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates), **and (v) experiments involving gene drive modified organisms (Section III-D-8).**

Appendix G-III-B. Arthropod Containment Guidelines, Version 3.2, 2019, and Addendum 1 Containment Practices for Arthropods Modified with Engineered Transgenes Capable of Gene Drive, 2022, American Committee of Medical Entomology, American Society of Tropical Medicine and Hygiene, Arlington, Virginia.

Appendix L – Recombinant Plants
Appendix L-III-C. Biological Containment Practices (Macroorganisms)
Appendix L-III-C-1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water. Containment for arthropods is described in the Arthropod Containment Guidelines and Addendum 1 Containment Practices for Arthropods Modified with Engineered Transgenes Capable of Gene Drive (see Appendix G-III-B).

Appendix M Animals:
Other types of animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in Appendices G and L. Containment for arthropods is described in the Arthropod Containment Guidelines and Addendum 1 Containment Practices for Arthropods Modified with Engineered Transgenes Capable of Gene Drive (see Appendix G-III-B).