

Proposed Revisions to the *NIH Guidelines*: Responses to Public Comments

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Overview

- **Revisions to *NIH Guidelines***
 - **Expansion of scope to cover research with synthetic nucleic acids**
 - **Changes to Section III-A-1: Transfer of drug resistance to microorganisms**
 - **Changes to Section III-E-1: Experiments involving less than 2/3 of genome of any eukaryotic virus**

Current Biosafety Guidance

- ***NIH Guidelines* are limited to synthetic DNA joined by recombinant methods**
 - Does not cover synthetic DNA that is synthesized *de novo*
 - Does not cover synthesized RNA viruses
- **Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL)**
 - Agent specific, not technology driven
 - References *NIH Guidelines* with respect to synthetic recombinant molecules

Synthetic Nucleic Acids (NAs) and Biosafety: U.S. Government Policy

- **National Science Advisory Board for Biosecurity (NSABB) recommendation: “need for biosafety principles and practices applicable to synthetic genomics”**
- **NSABB recommendations were considered through a trans-federal policy coordination process**
- **Recommendation on need for biosafety guidance accepted by U.S. Government with understanding that implementation would be through modification of the *NIH Guidelines* as appropriate**

Charge to the Recombinant DNA Advisory Committee

- Consider the application of the *NIH Guidelines* to experiments with synthetic nucleic acids
 - To what degree is this technology covered?
 - Does the scope need to be modified to capture synthetic biology research?
- Develop draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic nucleic acids

RAC Biosafety Working Group Roster

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Process to Date

- **Proposed revisions developed by the Biosafety Working Group and approved by the full RAC in March 2008**
- **Proposal published in Federal Register (FR) in March 2009 with opportunity for public comment**
- **Stakeholders Meeting to discuss public comments with experts on June 23, 2009**
- **RAC Biosafety WG meeting in October 2009 to consider public comments and propose revisions to certain sections of language proposed in the FR notice**

Revisions to Proposed Language FR Notice (March 2009)

- **In response to Public Comments the RAC Biosafety WG proposed revisions to the proposal published in March 2009**
 - **Section I-A Purpose and I-B Definition**
 - **Section III-F exemptions**
 - **Appendix M**
 - **Section III-A-1-a Experiments involving introduction of drug resistance into microorganisms**
 - **Section III-E-1 Experiments involving no more than 2/3 of the genome of any eukaryotic cell**

Proposed Language FR March 2009

Section I-B Definition

In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:

- (i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell,
- (ii) Synthetic nucleic acid molecules that are chemically, or by other means, synthesized or amplified nucleic acid molecules that may wholly or partially contain functional equivalents of nucleotides, or
- (iii) molecules that result from the replication of those described in (i) or (ii) above.

Section I-B Definition

Public Comments

- **Comment**
 - Please provide clarity on meaning of “functional equivalents of nucleotides.”

RAC Biosafety Working Group Discussions

- **Intent of including “functional equivalents of nucleotides,” was to capture molecules that had structures that are so different than a nucleotide that they would not be recognized as such and yet can perform equivalent function, e.g. transcription, translation**
 - **Chemically modified nucleotides that have a structure that is recognizable as being based on a nucleotide are already captured**

RAC Biosafety Working Group Recommendation

- **Recognition that such entities do not yet exist and therefore accurately defining an entity that does not exist is problematic.**
- **Remove “functional equivalents” language and clarify that definition will capture chemically modified nucleic acids.**

Proposed Revisions to Section I-B Definition

In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:

- (i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell,
- (ii) **Synthetic nucleic acid molecules that are chemically, or by other means, synthesized or amplified, including those that are chemically or otherwise modified but can base pair (bind) with naturally occurring nucleic acid molecules**
- (iii) molecules that result from the replication of those described in (i) or (ii) above.

Proposed Revisions to Section I-A Purpose

- The purpose of the NIH Guidelines is to specify the practices for constructing and handling: (i) Recombinant nucleic acid molecules, (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair (bind) with naturally occurring nucleic acid molecules, and (iii) cells, organisms and viruses containing such molecules.

Proposed Revisions to Section I-A Purpose and Section I-B Definition

RAC Discussion

Section III - F

Exempt Experiments

- *NIH Guidelines* currently exempt certain nucleic acid molecules from oversight due to low biosafety risk or because the nucleic acids either have not been modified from contemporaneous natural counterpart or the experiment parallels a natural process, e.g.
 - Certain recombinant nucleic acids that are being propagated in a host that is either the natural host for such nucleic acids or is a closely related prokaryotic or eukaryotic host

Proposed Language in FR (3/09)

III-F-1 Exemption

- Those synthetic nucleic acids that can not replicate, and that are not deliberately transferred into one or more human research participants (See Section III-C and Appendix M).

Exemption III-F-1

Public Comments

- Request for clarification as to whether “replication” would apply to plasmids lacking sequences needed to replicate in eukaryotic cells or cDNAs of + RNA viruses?
- Concern that synthetic integrating vectors should not be exempt from *NIH Guidelines*.
- Concern about exempting synthetic nucleic acids that could produce toxins or contain an oncogene, but there were also questions raised as to how to define an oncogene.

NIH Guidelines Section III-F-1

Exempt Experiments

The following recombinant and/or synthetic nucleic acid molecules are exempt from the *NIH Guidelines* and registration with the IBC is not required:

Proposed Revision to III-F-1

Section III-F-1

Those synthetic nucleic acids that:

- a) can neither replicate nor generate nucleic acids that can replicate in a living cell, and**
- b) are not designed to integrate into DNA, and**
- c) do not produce a toxin that is lethal for vertebrates at an LD50 < 100 nanograms, and**
- d) are not deliberately transferred into one or more human research participants (see Section III-C and Appendix M).**

Exemption III-F-1

Non-replicating Synthetic NAs and Human Gene Transfer

Question posed in March 2009 FR notice

- Are there classes of non-replicating molecules that should be exempt due to lower potential risks (e.g., antisense RNA, RNAi, etc.). If so, what criteria should be applied to determine such classes?**

Non-replicating Synthetic NAs: Human Gene Transfer

- **Comments provided by pharmaceutical and biotechnology companies and academic researchers.**
- **Discussion with experts at June 23rd public consultation.**
- **Proposal submitted by Oligonucleotide Safety Working Group**
 - **Consensus opinion developed by 70 pharmaceutical and regulatory professionals that discuss non-clinical issues and strategies to facilitate safe development of oligonucleotide drugs**

Proposal from Oligonucleotide Safety Working Group

Proposed that the following synthetic oligonucleotides should be exempt from RAC review:

- **Contain fewer than 100 nucleotides in total (single stranded, double stranded, or partially double stranded); AND**
- **Unable to integrate into the genome (i.e. do not contain known viral vector, transposable element or other known sequences designed to promote integration of the molecule into the genome); AND**
- **Cannot be replicated in cells (i.e. do not contain elements known to interact with either DNA or RNA polymerase); AND**

Proposal from Oligonucleotide Safety Working Group

Continued...

- **Do not comprise a gene (i.e. do not contain promoter / enhancer elements, transcription initiation elements or polyadenylation sequences designed to enable the molecule to be transcribed into mRNA); AND**
- **Cannot be reverse transcribed into DNA (i.e. cannot be recognized by reverse transcriptase); AND**
- **Cannot be translated into protein (i.e. do not contain elements that interact with ribosomal subunits); AND**
- **Have a transient effect (reversible in time, not permanent - must be re-administered to sustain effect).**

RAC Biosafety Working Group

Human Gene Transfer Recommendations

- **RAC BSWG considered all of the comments, including the Oligonucleotide WG proposal and the scientific literature.**
- **Concluded that given the experience with DNA oligos, which are well characterized in terms of biological risks, it is reasonable to exempt these from the requirements of Appendix M, including registration with OBA and RAC review.**

RAC Biosafety Working Group

Human Gene Transfer Recommendations

- **With respect to synthetic RNA constructs that can not replicate and are not contained in vectors, there is insufficient data regarding safety of use in human trials, including potential off-target and long-term effects.**
- **Recommended that for now these not be exempt but that this decision should be revisited in the future when additional data are available.**

RAC Biosafety Working Group

Human Gene Transfer Recommendations

- Exemption will be done under Appendix M and be similar to the current exemption for certain vaccine trials which are exempt from requirements for protocol submission, RAC review and reporting but require IBC review.

Proposed Addition to Appendix M (Human Gene Transfer)

Appendix M-VI-B

Human studies in which synthetic DNA nucleotides are deliberately transferred into one or more human research participants are exempt from Appendix M-1, *Requirements for Protocol Submission, Review and Reporting- Human Gene Transfer* if the DNA nucleotides meet the following criteria:

Proposed Addition to Appendix M (Human Gene Transfer)

Continued...

- **Contain fewer than 100 DNA nucleotides in total (single stranded, double stranded, or partially double stranded); AND**
- **Unable to integrate into the genome (i.e. do not contain known viral vector, transposable element or other known sequences designed to promote integration of the molecule into the genome) AND**

Proposed Addition to Appendix M (Human Gene Transfer)

Continued...

- **Cannot replicate in cells (i.e. do not contain elements known to interact with either DNA or RNA polymerase); AND**
- **Do not comprise a gene (i.e. do not contain promoter/enhancer elements, transcription initiation elements or polyadenylation sequences designed to enable the molecule to be transcribed into mRNA).**

Proposed Addition to Appendix M (Human Gene Transfer)



RAC Discussion





**Section III-A-1:
Experiments that Require
NIH Director Approval
(Major Actions)**



Current Section III-A-1-a: Major Action

- The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B, *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.

Proposed Language FR (3/09)

Section III-A-1-a

- **March 2009 Federal Register notice proposed to remove the criterion that if a microorganism has already acquired resistance to the drug being proposed for use in the laboratory the experiment does not require review under Section III-A-1**
 - Rationale: The current language in the NIH Guidelines may fail to capture a small set of experiments in which there is low level of resistance to a clinically useful drug but nonetheless use of that drug resistance marker raises public health concerns

Proposed Language FR (3/09)

Section III-A-1-a

- In addition, the March 2009 Federal Register notice clarified that the use of drugs other than the “first line” drug can still be a Major Action if:
 - The use of the drug may compromise the ability to control disease in other patient groups, such as children or pregnant women or
 - It is the primary drug available and used internationally, especially for a pathogen whose disease burden primarily lies outside the U.S.

Proposed Language FR (3/09)

New Section III-B-2

- Upon receipt and review of an application from an investigator, NIH/OBA may determine that a proposed experiment is equivalent to an experiment that has previously been reviewed by the NIH Director as a Major Action, including experiments approved prior to implementation of these changes. An experiment will only be considered equivalent if, as determined by NIH/OBA, there are no substantive differences in the experimental design or pertinent information has not emerged since submission of the initial III-A-1-a experiment that would impact on the biosafety or public health risks for the proposed experiments. If such a determination is made by NIH/OBA these experiments will not require review and approval under section III-A-1-a.

Section III-A-1-a

Public Comments

- **Concern that the removal of the “naturally acquired” language would overly expand the experiments requiring review and/or lead to multiple requests to OBA for clarification.**
- **Noted a lack of evidence that the current criteria have been ineffective in protecting public health.**
- **Noted that establishment of drug resistance, and in particular antibiotic resistance, is primarily driven by activities outside of the laboratory, i.e. agriculture and clinical practice.**

Section III-A-1-a

Public Comments

- Proposed that once there are reports of antibiotic resistance in the community and literature, even low level resistance, there are considerable number of resistant strains and proper containment in the labs is sufficient; further review not required.

Section III-A-1-a

Public Comments

- Agreed with OBA that that there may be certain experiments in which resistance is documented in the community but containment of this resistant organism needs to be considered carefully.
- Noted that not all IBCs have the same infectious disease expertise available to them and OBA should serve as a resource.

Proposed Revisions to Section III-A-1-a

- The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B, *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.
- **Consideration should be given to whether the drug-resistance trait to be used in the experiment would render that microorganism resistant to the primary drug available to and or indicated for certain populations, for example pediatric populations and pregnant women.**

Proposed Revisions to Section III-A-1-a

- **OBA will provide, following consultation as needed, a determination regarding whether a specific line of research involving the deliberate transfer of a drug resistance trait falls under Section III-A-1-a and therefore requires RAC review and NIH Director approval prior to initiation. An IBC may consult OBA regarding experiments that do not meet the requirements of Section III-A-1-a but nonetheless raise important public health issues and OBA will consult as needed with one or more experts, which may include the RAC.**

Proposed Revisions to Section III-A-1-a



RAC Discussion





**Section III-E-1:
Research In Tissue Culture with
no more than 2/3 of a Genome
of a Eukaryotic Virus**



Section III-E-1

Current *NIH Guidelines* Language

Recombinant DNA molecules containing no more than two thirds of the genome of any eukaryotic virus (all viruses from a single family being considered identical...) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments it must be demonstrated that cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures under Section III-D-3 . . should be used. The DNA may contain fragments of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Proposed Language FR (3/09)

Section III-E-1

Recombinant and synthetic nucleic acid molecules containing **no more than half of the genome of any one RG 3 or 4 eukaryotic virus** (all viruses from a single family being considered identical...) may be propagated and maintained in cells in tissue culture using BL1 containment (**as defined in Appendix G**) provided **there is evidence that the resulting nucleic acid in these cells are not capable of producing a replication competent nucleic acid.**

Proposed Language March 2009

Section III-E-1

For such experiments, it must be demonstrated that the cells lack helper virus for the specific families of defective viruses being used. If helper virus is present, procedures under Section III-D-3 should be used.

The nucleic acids may contain fragments of the genome of viruses from more than one family but each fragment shall be **less than** $\frac{1}{2}$ of a genome.

Rationale for Proposed Language (3/09)

- Proposal required that the size of the genome deletion be increased from 1/3 to greater than 50% of the genome of any single family of virus
 - Concern that synthetic viruses might be created containing multiple segments of NAs from a family of viruses or different viruses and yet be infectious
 - Questions raised by research with herpes viruses with multiple deletions
- Also required the PI to provide evidence that the remaining nucleic acid in these cells is not capable of producing a replication competent virus (RCV) in addition to lacking helper virus
 - Recognition that helper virus function may carry risk of rescue

Section III-E-1

Public Comments

- Three of the five public comments submitted to OBA supported the proposed changes.
- One respondent proposed that the criterion for lowering containment should be based on the nature of a functional impairment (*e.g.* an irreversible biological defect) rather than the physical extent of a genomic deletion that achieves a similar functional outcome.
- Another respondent noted that a requirement for a 50% deletion would force VEE-based vaccine work (*i.e.* replicons) to be conducted at BL3. Currently under the 2/3 rule, BL2 containment is permissible for replicon work.

Section III-E-1

Biosafety Working Group Discussion

- **Considered proposals at the June 2009 Stakeholders meeting as a springboard to develop criteria based on impairments of structural or functional genes in addition to impairments based on genome deletions.**

Section III-E-1

Proposed Revisions

Recombinant and synthetic nucleic acid molecules containing:

- 1) no more than half of the genome of any RG3 or RG4 eukaryotic virus OR
- 2) nucleic acid molecules from any Risk Group eukaryotic virus containing a complete deletion in one or more essential viral capsid, envelope or polymerase genes required for cell-to-cell transmission of viral nucleic acids (all viruses from a single family being considered identical) . . . may be propagated and maintained in cells in tissue culture using BL1 containment (as defined in Appx G) provided there is evidence that the resulting nucleic acids in these cells are not capable of producing a replication competent virus. For such experiments, it must be demonstrated that the cells lack helper virus for specific families of defective viruses being used . . .

Section III-E-1

Proposed Revisions

For retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis, a minimum of BL2 containment is required.

Section III-E-1

Proposed Revisions

- **Criteria 2 requires a complete deletion of one or more genes thereby excluding point mutations, frame shifts or partial gene deletions that could be reversed**
 - This should prevent recombination events that could occur between homologous DNA sequences and impaired viruses bearing partial gene deletions
- **Requirement for BL2 for lenti and retroviruses addresses biosafety concerns regarding the risk of insertional mutagenesis and is consistent with the existing lentiviral vector guidance**

Section III-E-1

Proposed Revisions



RAC Discussion



PUBLIC COMMENTS

