Proposed Changes to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*

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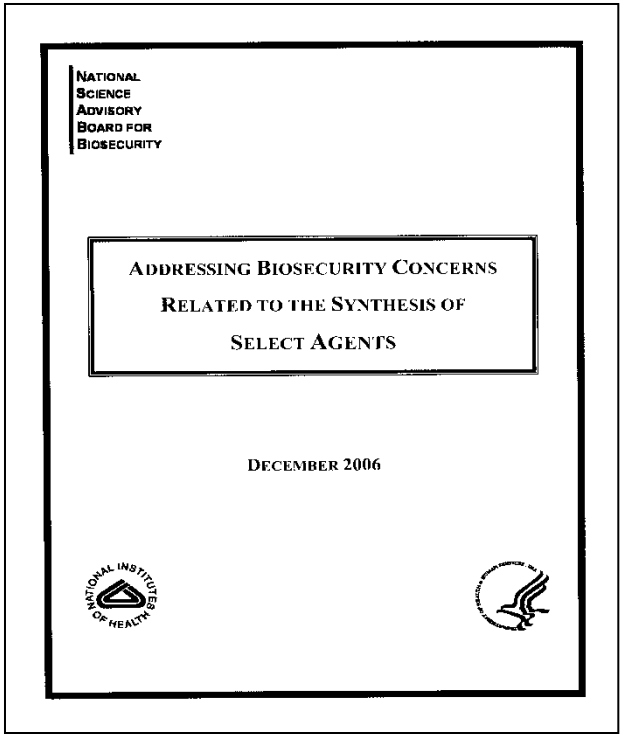
Proposed Revisions to the *NIH Guidelines*

- **Research with Synthetic Nucleic Acids**
  - ***What will be covered and what won't***
  - ***Risk Assessment***
- **Research with Partial Viral Genomes in Tissue Culture**
- **Updates to the Classification of Human Agents on the Basis of Hazard (Risk Groups)**

National Science Advisory Board for Biosecurity (NSABB) Report



http://oba.od.nih.gov/biosecurity/biosecurity_documents.html

NSABB Findings

- **Some practitioners of synthetic genomics are:**
  - **Educated in disciplines that do not routinely entail formal training in biosafety; and**
  - **Uncertain about when to consult an Institutional Biosafety Committee (IBC).**
- **There is a need for biosafety principles and practices applicable to synthetic genomics.**

Current Biosafety Guidelines

- ***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**

NIH Recombinant DNA Advisory Committee (RAC)

- **Considered the application of the *NIH Guidelines* to synthetic biology**
  - **To what degree is this technology covered?**
  - **Does the scope need to be modified to capture more explicitly synthetic biology research?**
- **Developed recommendations regarding principles and procedures for risk assessment and management of research involving synthetic nucleic acids (NAs)**

Synthetic Nucleic Acids Current Language

- ***NIH Guidelines*** **define Recombinant DNA as:**
  - **Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or**
  - **Molecules that result from the replication of those described above**

Approach to Identifying Research with Synthetic NAs for Inclusion under the *NIH Guidelines*

- **Capture the same products made by synthetic techniques that are currently covered under the *NIH Guidelines* for recombinant DNA research, provided the same biosafety concerns are raised**
  - **Level of review should be based on risk not technique**

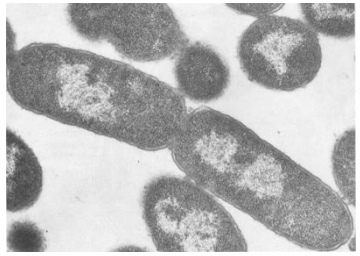
Proposed Definition of Recombinant and Synthetic Nucleic Acid Molecules

(i) Molecules that are constructed by joining nucleic molecules and that can replicate in a living cell, i.e. recombinant nucleic acid molecules,

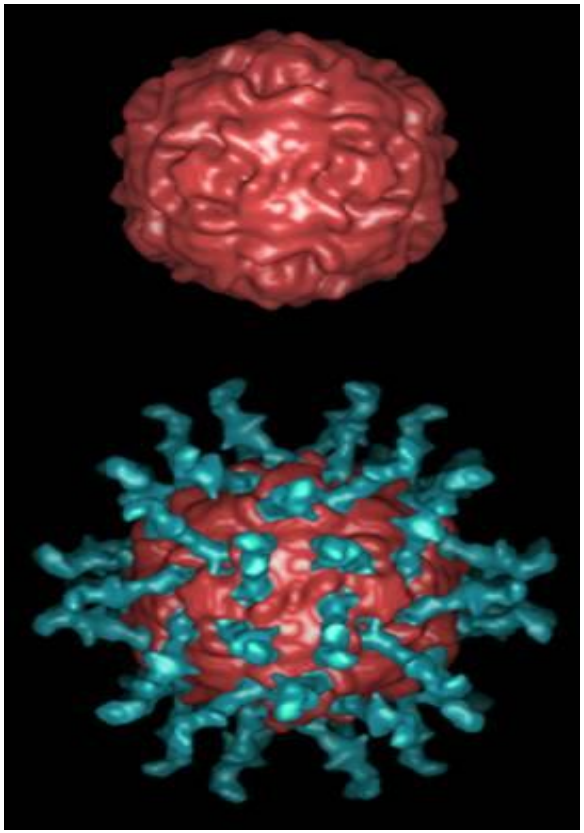
(ii) Molecules that are chemically, or by other means, synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e. synthetic nucleic acids, or

(iii) Molecules that result from the replication of those described in (i) or (ii) above.

What is Covered under the *NIH Guidelines*?



What is Not Included under the *NIH Guidelines*?



The following research is exempt:

- **Research with NA molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more segments may be a synthetic equivalent**
- **Synthetic reconstruction of existing viruses would not be covered**

Replication: A Key Characteristic of Recombinant Molecules

- **Recombinant DNA molecules are those that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that <u>can replicate in a living cell.</u>**

- **The ability to replicate was seen by the RAC as a key characteristic of recombinant research that warranted oversight under the *NIH Guidelines*.**

What is the Biosafety Risk of Non-replicating Synthetic NAs?

- **Exposure in the lab to a low dose of non-replicating synthetic nucleic acid sequence is considered low risk**
  - **No replication even if the NAs enter a cell**
  - **No ability to spread in the environment if released**
  - **NAs are not toxic in and of themselves**

Basic Research with Synthetic NAs under the *NIH Guidelines* (Proposed)

- **Research with synthetic nucleic acids that can neither replicate nor generate nucleic acids that can replicate in any living cell will be exempt**

UNLESS

- **They are designed to integrate into the genome**
- **Can produce a toxin with LD50 < 100 ng/kg**
- **Are used in human gene transfer research**

Human Gene Transfer (HGT) Under the *NIH Guidelines* – Is Replication the Key Issue?

- **Clinical safety considerations often independent of vector replication including:**
  - **transgene effects,**
  - **insertional mutagenesis, and**
  - **immunological responses.**

Human Gene Transfer (HGT) Under the *NIH Guidelines* – Is Replication the Key Issue?

- **Doses and routes used in human gene transfer potentially increase risks compared to those anticipated for inadvertent lab exposure**
- **Human gene transfer often raises unique scientific, medical and ethical issues that warrant transparent oversight**

Is the Use of a Vector a Key Consideration for Oversight of HGT?

- **OBA received public comments that delivery of recombinant or synthetic NAs by a viral, bacterial or plasmid vector is human gene transfer**
- **Considerable debate by the RAC as to whether synthetic RNA and DNA not delivered by a viral, plasmid or bacterial vector should be under the *NIH Guidelines***

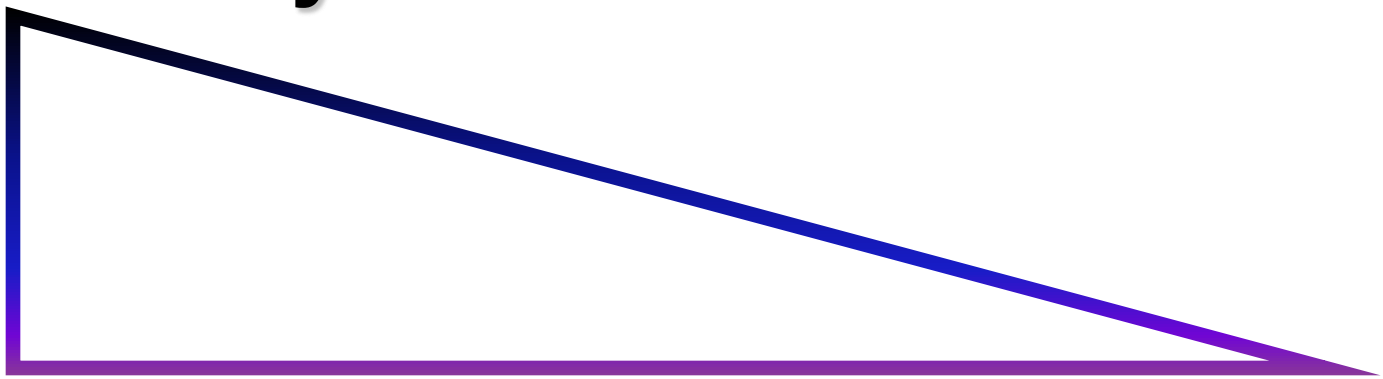
Human Gene Transfer Using Synthetic NA Under the *NIH Guidelines* (Proposed)

Clinical protocols using synthetic DNA or RNA will be subject to the *NIH Guidelines* if the investigational agent:

- **Contains greater than 100 nucleotides or base pairs in total; or**
- **Has characteristics that enable integration into the genome; or**
- **Is known to replicate in a cell; or**
- **Is known to be transcribed or translated**

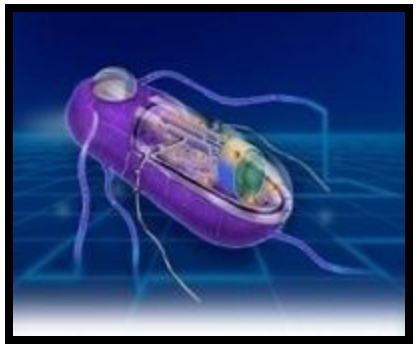
Risk Assessment for Research with Synthetic NA

Data to support a Biosafety Risk Assessment

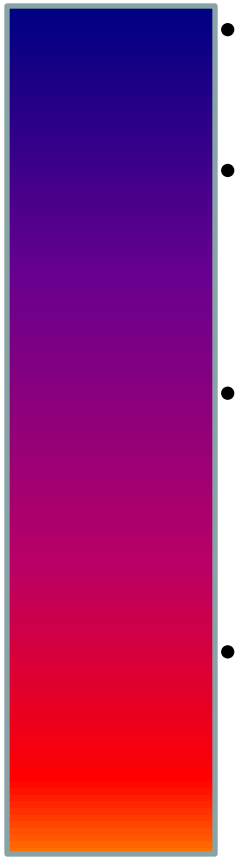


Known Agents

Novel or Uncharacterized Agents



Risk Assessment - *NIH Guidelines*



- **Risk Group (RG)1 -** Agents that are not associated with disease in healthy adult humans
- **RG2 -** Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
- **RG3 -** Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
- **RG4 -** Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *are not usually* available (high individual risk and high community risk)

Risk Assessment for Synthetic NA

(Proposed)

- **Risk Assessment is not fundamentally different from that for recombinant DNA research; however**
  - **As the technology moves forward, chimeras may be generated for which the parent organism is not obvious**
  - **Factors to be considered:**
    - **percent of genome contributed by each of multiple parental agents**
    - **Predicted function or intended purpose of each sequence**

Risk Assessment for Synthetic NA

(Proposed)

- **Assume the sequence will function as it does in the original host**
- **Consider the possibility that synergism between sequences and transgenes may result in a novel organism whose risk profile is higher than that of the contributing sequences or organisms**

Research with Partial Genomes of Eukaryotic Viruses in Tissue Culture

Containment for Certain Research with Partial Viral Genomes in Tissue Culture

- ***NIH Guidelines*** **currently allows certain tissue culture experiments to be initiated upon registration with the Institutional Biosafety Committee (IBC) and conducted at Biosafety Level 1 containment IF**
  - **No more than 2/3rds of full viral genome is present, AND**
  - **The cells lack helper virus**

Initial Proposal (March 2009)

- **Reduce the two thirds requirement to <u>ONE-HALF</u>**
  - **concern that it may be possible to generate a functional virus containing less than 2/3 of the genome, potentially using synthetic constructs**

Public Comment on Initial Proposal

- **Research has been safely conducted for many years under this section with viruses that contain more than 50% of the genome but less than 66%, e.g. viral replicon particles of Venezuelan Equine Encephalomyelitis**
- **Support for a scientific basis for reduction in containment, not just a quantitative standard**

Final Proposed Changes

Tissue culture experiments with the following constructs can qualify for a reduction in containment:

- **Tissue culture experiments using RG3 and 4 agents provided less than one-half of any viral genome is present,* OR**

Research with 50% or less of the genome of a RG2 virus is exempt from the *NIH Guidelines

Final Proposed Changes, (2)

For any RG agent, if one or more essential viral capsid, envelope or polymerase gene(s) required for cell-to-cell transmission of viral nucleic acids is deleted,

AND

- **There must be evidence that the resulting nucleic acids are not capable of producing replication competent virus in a cell line that would normally support replication of wild-type virus**

Final Proposed Changes (3)

Evidence that Must be Submitted to IBC

- **When reduction in containment is based on deletions of the capsid, envelope, or polymerase genes, the PI must provide evidence that demonstrates a complete deletion of the genome sequence such that it is not possible to rescue critical functions through homologous recombination**

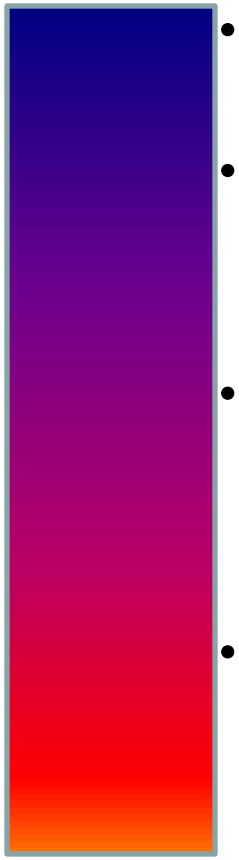
Further Discussion of Section III-E-1

- **Further discussion of this proposal will occur at the RAC Meeting on June 9, 2011**

- **Webcast: http://oba.od.nih.gov/rdna_rac/rac_meetings.html**

Proposed Updates to the Classification of Human Etiologic Agents on the Basis of Human Hazard- i.e. Risk Groups

Risk Assessment - *NIH Guidelines*



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Proposed Updates to Risk Groups

- *NIH Guidelines* current classification of organisms into risk groups does not include many attenuated strains used in recombinant research (Appendix B)

- Some RG3 viruses are not specifically listed as RG3 viruses, e.g. West Nile Virus

Proposed Updates to Risk Groups

- As containment under the *NIH Guidelines* is often determined largely by the RG for an organism it is important to list these organisms in their appropriate RG
- A risk assessment for containment for many of these attenuated strains and RG3 viruses has been done by NIH and CDC in developing the CDC/NIH BMBL

Proposed Updates to Risk Groups

The following are proposed to be added to the list of RG2 Bacteria:

- ***Coxiella burnetii*** **Nine Mile strain, plaque purified clone 4**
- ***Francisella tularensis*** **subspecies**
  - ***novicida*****, Utah 112**
  - ***holartica*** **LVS**
  - **biovar tularensis strain ATCC 6223 (also known as strain B38)**

- ***Yersinia Pestis, pgm$^{(-)}$ and lcr$^{(-)}$***

Proposed Updates to Risk Groups, Cont…

The following are proposed to be added to the list of RG2 Viruses:

- ***Chikungunya vaccine strain 181/25***
- ***Junin Virus candid #1 vaccine strain***

The following are proposed to be added to the list of RG3 viruses:

- ***SARS-associated Coronavirus* (*SARs-CoV*)**
- ***Chikungunya virus***
- ***West Nile Virus***

Next Steps

Proposed Changes for Research with Synthetic NA and Partial Viral Genomes:

- **Final Federal Register (FR) notice is undergoing agency review**
- **As full implementation will necessitate changes in IBC procedures, OBA is considering a grace period before full compliance required**

Next Steps

Proposed Changes to Risk Group Classification:

- **Considered a "minor action" under the *NIH Guidelines* and does not require public comment, but a FR notice will be published prior to implementation with opportunity for public comment**

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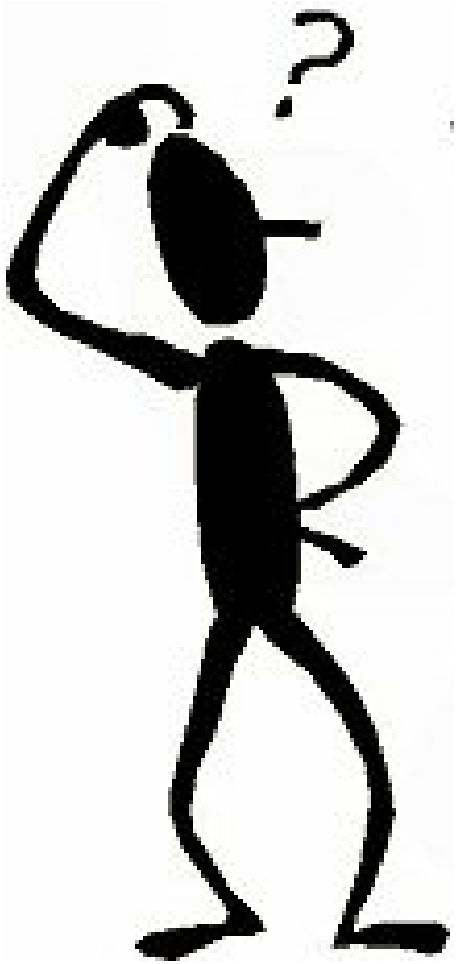

Questions?