



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

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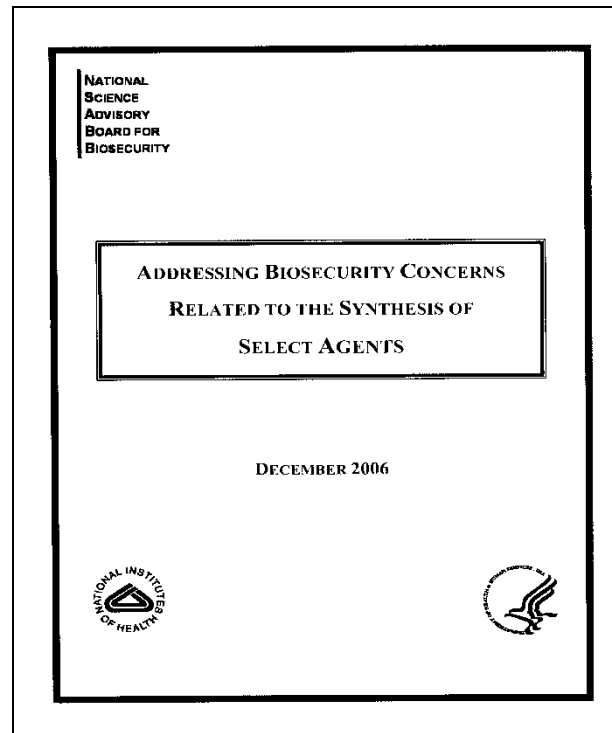


## **Proposed Revisions to the *NIH Guidelines***

- **Research with Synthetic Nucleic Acids**
  - *What will be covered and what won't*
  - *Risk Assessment*
- **Drug Resistance and Microorganisms – II-A-1**
- **Research with Partial Viral Genomes in Tissue Culture – II-E-1**
- **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](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 acid molecules and that can replicate in a living cell, i.e. recombinant nucleic acid molecules,**
- (ii) 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 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.**

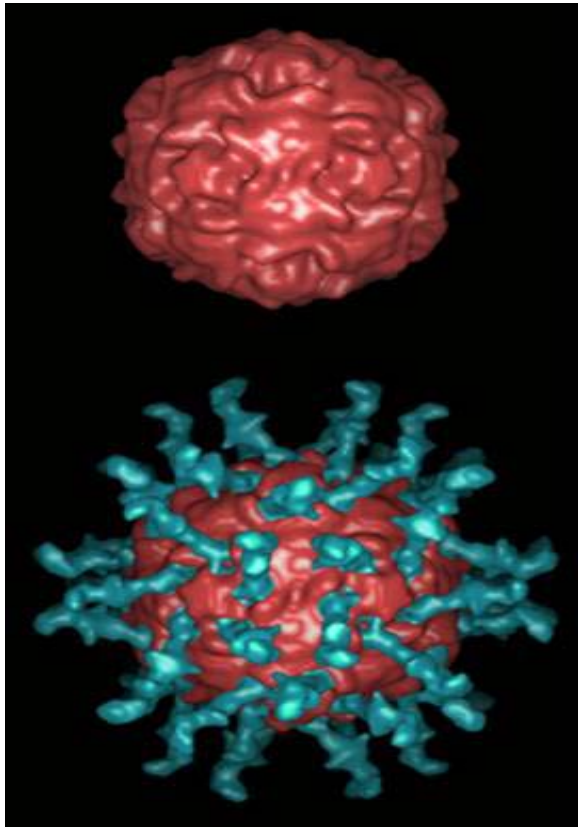


# What is Covered under the *NIH Guidelines*?





# 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 can replicate in a living cell.
- 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:

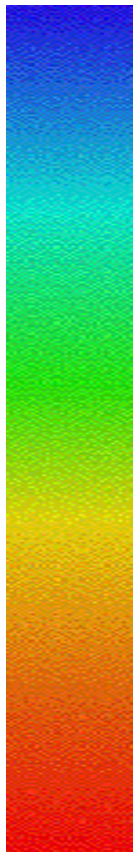
- ❑ Contains greater than 100 nucleotides or base pairs in total; or
- ❑ Has characteristics that enable integration into the genome; or
- ❑ Is known to replicate in a cell; or
- ❑ Is known to be transcribed or translated



**RISK ASSESSMENT UNDER THE *NIH*  
*GUIDELINES* AND SYNTHETIC  
NUCLEIC ACID RESEARCH**



# Risk Assessment - *NIH Guidelines*

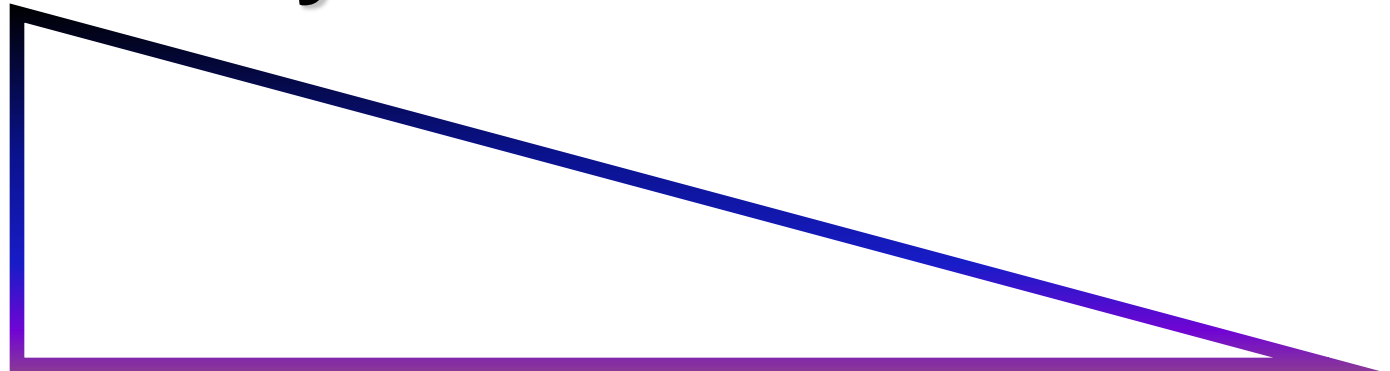


- **Risk Group (RG)1** - Agents that are not associated with disease in healthy adult humans
- **RG2** - Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
- **RG3** - Agents that are associated with serious 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 Research with Synthetic NA

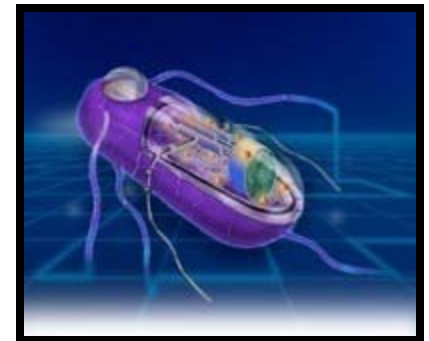
Data to support  
a Biosafety Risk  
Assessment



Known Agents



Novel or Uncharacterized  
Agents





# **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**



# **Introduction of Drug Resistance into Microorganisms**

## **Section III-A-1-a**





## **Section III-A-1-a Major Actions**

- **Introduction of drug resistance into a microorganism that:**
  - **“Not known to acquire the trait naturally” and**
  - **“Acquisition of the drug resistance could compromise the use of the drug to control disease” in humans, animal and plants.**
- **Requires RAC review and NIH Director approval.**



# Proposed Language

- The deliberate transfer of a drug resistance trait to microorganisms, ~~that are not known to acquire the trait naturally~~, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture, will be reviewed by RAC



## Proposed Language

- ❑ **Even if an alternative drug or drugs exist for the control or management of disease, it is important to consider how the research might affect the ability to control infection in certain groups or subgroups by putting them at risk of developing an infection by such microorganism for which alternative treatments may not be available. Affected groups or subgroups may include, but are not limited to: children, pregnant women, and people who are allergic to effective alternative treatments, immunocompromised or living in countries where the alternative effective treatment is not readily available.**



## Final Proposed Language

- ❑ **The deliberate transfer of a drug resistance trait to microorganisms, that are not known to acquire the trait naturally, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture, will be reviewed by RAC.**
- ❑ **Consideration should be given as to whether the drug resistance trait would render the microorganism resistant to the primary drug available for certain populations, for example children and pregnant women**



## Proposed Section III- B

- **Once an experiment is reviewed by the RAC and NIH under this section, equivalent experiments may be reviewed by OBA if there are no substantive changes in the experiment, and new pertinent information has not emerged since submission of the initial III-A-1-a experiment that would change the biosafety or public health considerations for the proposed experiments.**



# **Research with Partial Genomes of Eukaryotic Viruses in Tissue Culture**

## **III-E-1**



# 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 ONE-HALF
  - concern that it may be possible to generate a functional virus containing less than  $2/3$  of the genome, potentially using synthetic constructs
- Added requirement to document lack of replication competent virus





# **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 functional basis for reduction in containment, not just a quantitative standard**



## Section III-E-1 – Proposal (April 2010)

An investigator can initiate work at BL1 containment in tissue culture upon notification of the IBC if:

1) No more than half of the eukaryotic viral genome is present

**OR**

2) There is a complete deletion in one or more **essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission** of viral nucleic acids and the investigator provides the IBC with evidence such as sequence or other appropriate data to demonstrate that there is a complete deletion of genetic sequence such that these functions can not be rescued through homologous recombination.



## **Section III-E-1 – Proposal** (April 2010)

**AND**

**In both situations there must be evidence that:**

- 1. The resulting nucleic acids are not capable of producing a replication competent virus in a cell line that would normally support replication of the wild-type virus**
- 2. There is no helper virus present**

**Notwithstanding above, a minimum of BL2 containment is required for experiments with retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.**



## **Section III-E-1**

# **Additional Biosafety Concerns**

- **RAC review of research to create a defective RG4 agent raised questions about whether research with RG4 viruses is appropriate under this section as prior IBC review not required.**
  - The possibility of a rare event resulting from homologous and/or non-homologous recombination could result in the rescue of a potentially lethal virus at lower containment.



## **Section III-E-1**

# **Additional Biosafety Concerns**

- **Documenting that rescue of replication competent virus would not occur, presents considerable challenges including:**
  - **Establishing the sensitivity of biological and physical detection methods for replication competent virus.**
  - **Designing a statistically rigorous experiment that could demonstrate the strength of negative data given the likelihood of low incidence rates associated with such events.**

**Exclude Risk Group 4 Viruses from Section III-E-1?**



## **Section III-E-1**

# **Additional Biosafety Concerns**

- **Further discussion is needed regarding the criteria for RG3 and RG2 virus research that should be subject to Section III-E-1**
  - **Articulate guidance for investigators regarding the data needed to lower containment with defective RG2 and RG3 viruses in light of the differences in risks of recombination among different viruses and the cell lines being used to propagate and maintain them**



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



# Risk Assessment - *NIH Guidelines*

- **Risk Group (RG)1** - Agents that are not associated with disease in healthy adult humans
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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*<sup>(-)</sup> and *Icr*<sup>(-)</sup>



## **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 III-A-1-a

- 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 Section III-E-1 – Defective Viral Genomes in Tissue Culture:**

- **A new FR notice will be published for comment**



# 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

