



Updates to the *NIH Guidelines for Research Involving Recombinant DNA*

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OVERVIEW

- ❑ **Impetus for Change and Process to Date**
- ❑ **Proposed Changes to the *NIH Guidelines***
 - **Synthetic Nucleic Acids**
 - **Section III-E-1: the “2/3” Rule**
 - **Section III-A-1: Major Actions**
 - **Section III-E-3: Transgenic Mice**



National Science Advisory Board for Biosecurity

- ❑ **Advises the Secretary of the Department of Health and Human Services, the NIH Director and the heads of 15 departments and agencies with a role/interest in life sciences research**
- ❑ **Charged with recommending strategies for mitigating the potential for misuse of dual use biological research**
 - **Consider both national security concerns and the needs of the research community**



**NATIONAL
SCIENCE
ADVISORY
BOARD FOR
BIOSECURITY**

**ADDRESSING BIOSECURITY CONCERNS
RELATED TO THE SYNTHESIS OF
SELECT AGENTS**

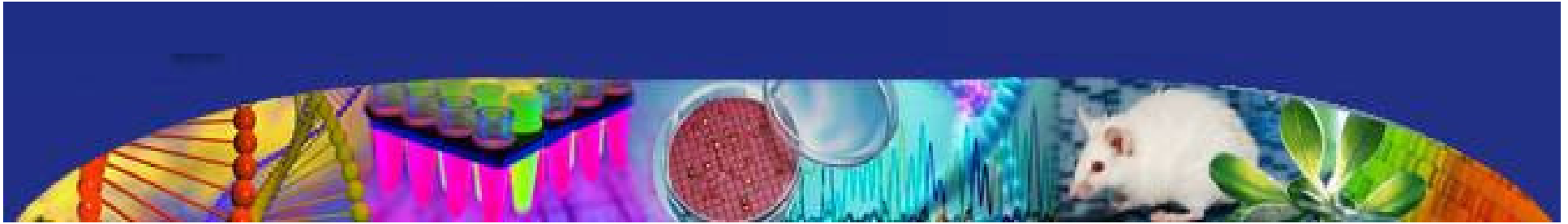
DECEMBER 2006





NSABB Findings

- ❑ **Some practitioners of synthetic genomics/biology 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/biology.**



Implementation of NSABB Recommendations

- ❑ **NSABB recommendations were considered through a trans-federal policy coordination process**
 - **Led by the White House Homeland Security Council and Office of Science and Technology Policy**
- ❑ **Recommendation on need for biosafety guidance accepted by USG with understanding that implementation would be through modification of the *NIH Guidelines* as appropriate**



NIH Guidelines

Definition of Recombinant DNA

- 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



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



Recombinant DNA Advisory Committee

- ❑ **Consider 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 synthetic biology research?
- ❑ **Develop draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic biology**



Review Process

- ❑ **Initial proposal developed by a subgroup of the RAC, the Biosafety Working Group**
- ❑ **Proposed revisions published in March 2009 *Federal Register* with opportunity for public comment**



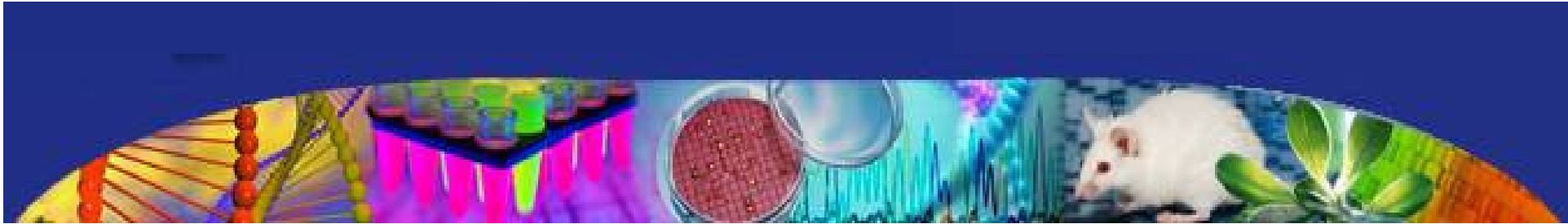
Review Process, cont. . .

- NIH Public Consultation on Proposed Changes to the NIH Guidelines, Arlington VA June 23, 2009**
 - http://oba.od.nih.gov/rdna_rac/rac_pub_con.html**
- Proposed revisions reviewed and approved by full the RAC at their public meeting in December 2009**



Overall Approach

- ❑ 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 based on risk not technique
- ❑ Develop a risk management framework that is based on the current science and what appears to be feasible in the foreseeable future
- ❑ Recognize that all not all future scientific developments can be anticipated, so that the *NIH Guidelines* will need periodic review and updating



Section I-B. Definition of Recombinant and Synthetic DNA

- (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 with naturally occurring nucleic acid molecules, or**
- (iii) molecules that result from the replication of those described in (i) or (ii) above.**



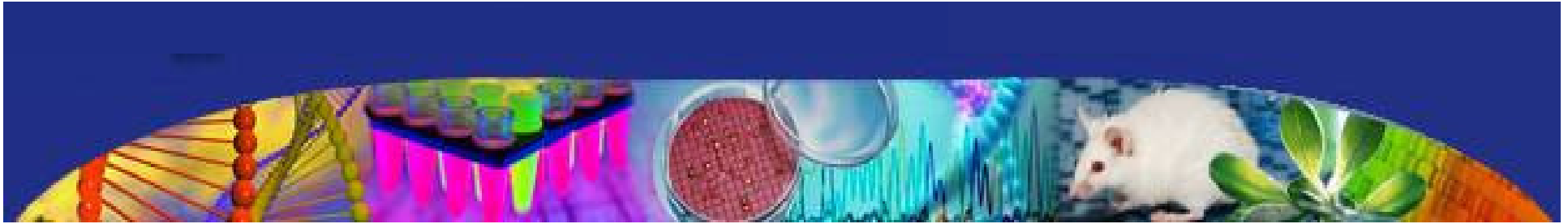
Replication: a Unique Risk

- ❑ **The ability to replicate is one of the unique risks of recombinant DNA molecules**
 - **Potential ability to propagate in the lab, in exposed laboratory workers, and the environment**
- ❑ **Are the risks of non-replicating synthetic molecules comparable to rDNA?**
 - **For basic research**
 - **For clinical research**



Non-replicating Synthetic NAs: Basic Research

- **Exposure in the lab to a low dose of non-replicating synthetic nucleic acid sequence is considered low risk**
 - **Limited because even if the NAs enter a cell they cannot replicate and spread**
 - **Could not spread in the environment if released**
 - **Exposure similar to that of a chemical exposure; however nucleic acids are not toxic in and of themselves**



Non-Clinical Nucleic Acid Research

Proposed Exemption for research with Synthetic NA that:

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



Human Gene Transfer

- ❑ Many human gene transfer trials use replication incompetent vectors; however, safety risks often arise from other factors that are not dependent on replication including transgene effects, insertional mutagenesis, and immunological responses
- ❑ Doses used in human gene transfer considerably higher compared to that anticipated for inadvertent lab exposure
- ❑ Human gene transfer often raises unique scientific, medical and ethical issues that warrant transparent oversight



Non-Vector vs Vector Constructs

- ❑ **Agreement by RAC members and public comments that vector constructs are human gene transfer, whether made synthetically or by recombinant means**
- ❑ **Considerable debate by RAC as to whether to include synthetic RNA and DNA not delivered by a traditional viral or bacterial vector**



Question posed in March 2009 FR

- **Are there classes of non-replicating molecules that should be exempt from the *NIH Guidelines* under III-C-1 due to lower potential risks (e.g., antisense RNA, RNAi, etc.). If so, what criteria should be applied to determine such classes?**



Public Comments- March 2009 Proposal

- **Urged RAC and OBA to differentiate synthetic RNA and DNA oligonucleotide agents from gene transfer agents that use vectors based on the following characteristics:**
 - **short half-life with more predictable pharmacokinetics**
 - **lack of ability to integrate into the genome**
 - **lack of replication or potential for inadvertent replication due to mobilization or recombination**
 - **lack of a transgene for coding a protein**



Oligo Safety Working Group* Proposed Exemption

- ❑ **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**

* OSWG is an independent consortium of over 90 pharmaceutical and regulatory professionals



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).**

Spectrum of Biologic Therapies

Long-Term Replacement of Function of Defective Gene (RV-ADA gene for ADA-SCID)

Designing Tumor Specific T cells (Chimeric Antigen Receptors for cancer)

Regulation of Gene Expression (plasmid zinc finger-transcription factor to upregulate VEGF-A for PAD)

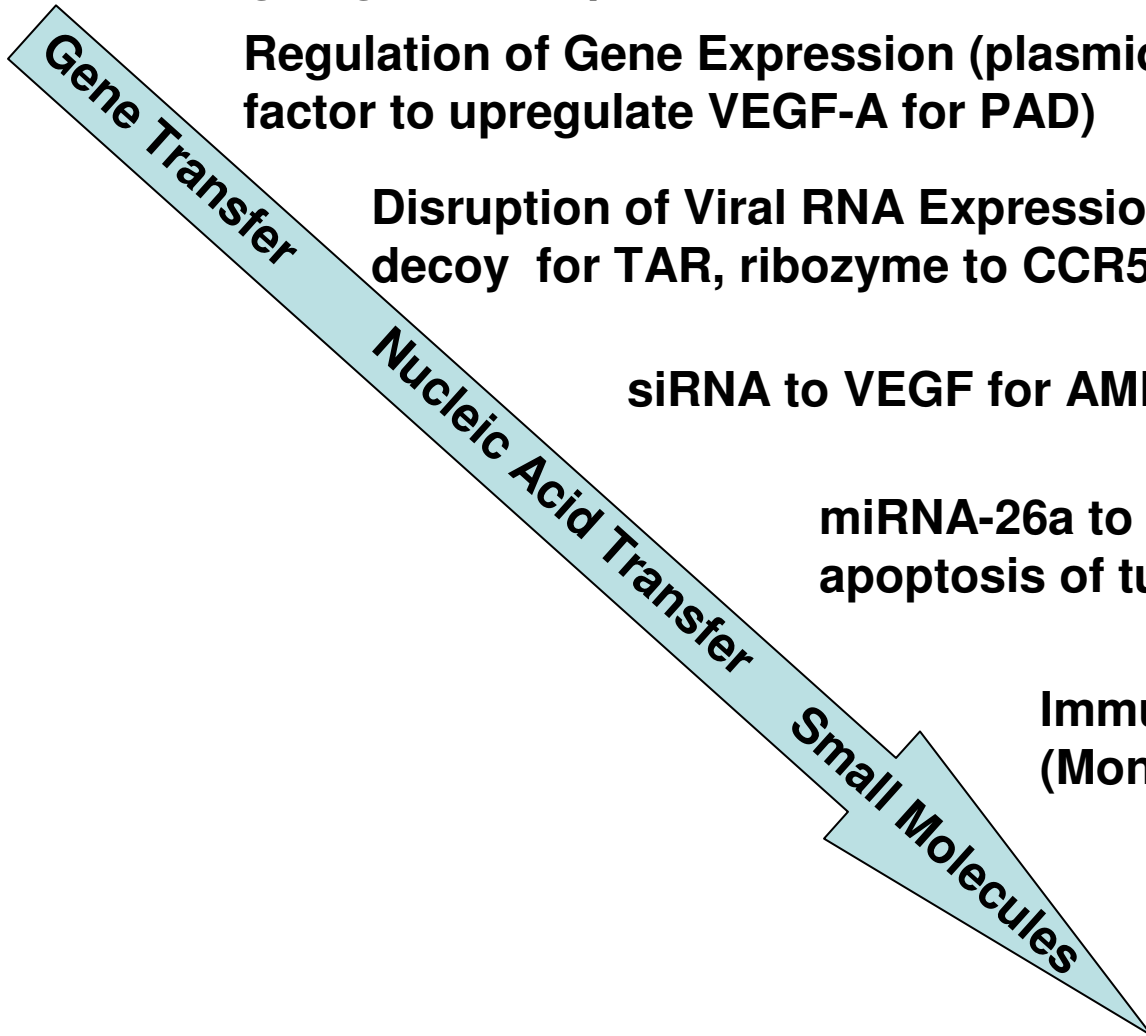
Disruption of Viral RNA Expression (LV-shRNA to tat/rev, RNA decoy for TAR, ribozyme to CCR5 for HIV)

siRNA to VEGF for AMD

miRNA-26a to inhibit Cyclin D2/E2 for apoptosis of tumor cells

Immunotherapeutic drugs
(Monoclonal Antibodies, Gleevec)

Recombinant Proteins
(Insulin)





RAC Recommendation re: DNA Oligonucleotides

- Mature Field**
- Mechanisms of action well characterized**



RNA Oligonucleotides

- **Relatively new field, clinical trials ongoing but rapidly expanding applications**
- **siRNA and miRNA have pleiotropic effects that are conserved across species**
 - **Individual miRNAs have been shown to suppress the production of hundreds of proteins (Baek, D., *et. al.*, 2008; *Nature* 455, 64)**



Significance of these preclinical findings?

- **RNAi for macular degeneration was result of immune stimulation of toll-like receptors (Kleinman, M., *et. al.*, 2008; *Nature* 452:7187).**
- **shRNAs led to a fatal toxicity due to unanticipated competition with endogenous miRNA processing (Grimm, D. *et. al.*, 2008; *Nature* 441: 537).**
- **Administration of siRNA targeting macrophage migration inhibitory factor (MIF), a cytokine with well described roles in cell proliferation, tumorigenesis, and angiogenesis, unexpectedly led to enhanced proliferation of breast cancer cells rather than the expected apoptosis (Armstrong, M. *et al.*, 2008; *J. Immunology* 180:7125).**



Could epigenetic changes have long term clinical consequences ?

- **Can siRNA and miRNA lead to long-term gene silencing** (Hawkins, P., *et. al.*, 2009 *Nucleic Acid Res.* 37(9):2984; Kim, D. *et. al.* 2009; *PNAS* 105:16230).



Weighing the Evidence



Clinical trials without evidence of unexpected toxicity or long-term adverse effects

Emerging basic research highlighting the potential limits of our understanding



Next Steps

- ❑ **Revisit the data at the June 17, 2010 Meeting of the Recombinant DNA Advisory Committee, Bethesda MD**
- ❑ **Webcast:**
http://oba.od.nih.gov/rdna_rac/rac_meetings.html



RISK ASSESSMENT UNDER THE *NIH GUIDELINES*





Risk Groups (RG)

- **RG1** 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

- ❑ Starting point for the Risk Assessment (RA) is the non-recombinant “parent” organism
- ❑ Containment may be raised or lowered depending upon the recombinant agent factors and manipulation:
 - Virulence
 - Pathogenicity
 - Infectious Dose stability
 - Route of Spread
 - Gene Product effects
 - Biological containment



Risk Assessment for Synthetic NAs

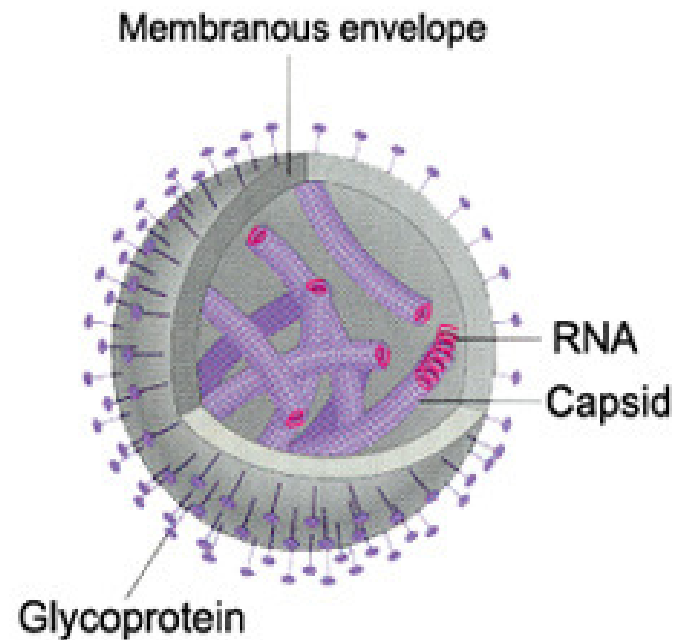
- **RA is not fundamentally different; however**
 - **As the technology moves forward, chimeras may be generated for which the parent organism is not obvious**
 - **RA should consider the organism(s) from which the sequences were derived and the function of those sequences**
 - **It may be prudent to first consider the highest risk group classification of any agent sequence in an organism made from sequences from multiple sources**

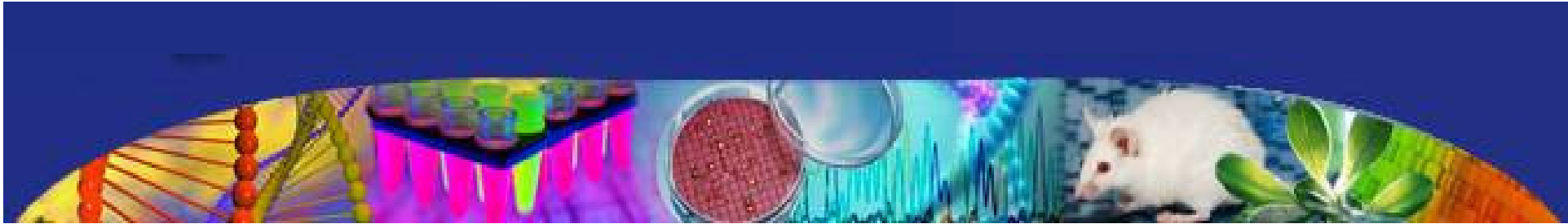


- **Other factors to be considered:**
 - **% of genome contributed by each of multiple parent agents**
 - **Predicted function or intended purpose of each sequence**
- **Assume the sequence will function as does in the original host**
- **Consider the possibility that synergism between sequences and transgenes may result in an organism whose risk profile is higher than that of the contributing sequences or organisms**



Section III-E-1 Experiments in Tissue Culture with Partial Viral Genomes





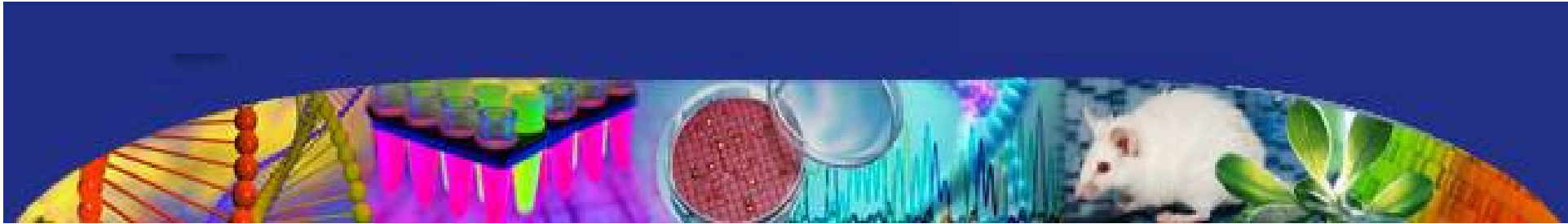
Section III-E-1: Tissue Culture Experiments

- ❑ **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 if**
 - **It is demonstrated that the cells lack helper virus for the specific Families of defective viruses being used.**
- ❑ **The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.**



RAC Review of III-E-1

- ❑ **Concern that synthetic viral agents derived from multiple sources might be able to function with less than 2/3 genome present, therefore**
 - **The 2/3 genome proposed to be changed to 1/2**
- ❑ **Generation of replication competent virus could arise from mechanisms other than presence of helper virus**
 - **Proposed to amend this section to require a demonstration that the preparation(s) are free of replication competent virus which may be generated by homologous recombination with endogenous proviruses or the presence of helper virus.**



Section III-E-1 Proposed March 2009

- **BL-1 containment permitted for experiments involving risk Group 3 and 4* viruses with less than one-half of any eukaryotic viral genome provided evidence is also submitted attesting that:**
 - **The resulting NA molecules in these cells are not capable of producing a replication competent virus and**
 - **The cells lack helper virus for the specific Families of defective viruses being used**

* Risk Group 1 and 2 viruses with less than one-half of the genome are exempt from *NIH Guidelines per Appendix C-1*



Section III-E-1 – FR Public Comments

- ❑ 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).
- ❑ Another respondent noted that a requirement for a 50% deletion would force VEE–based vaccine work (*i.e.* replicons) to be conducted at BL3.



Section III-E-1, cont. . .

- ❑ **The RAC considered a number of proposals**
- ❑ **Decision made to include criteria based on impairments to structural or functional genes in addition to a quantitative standard**



Section III-E-1 Revised – cont. . .

- ❑ **Well characterized viruses can be safely disabled by removal of certain critical genes – i.e. capsid, envelope and polymerase genes – that are essential components for replication and cell-to-cell transmission of infectious virions.**
 - **Deletion of gene must be complete; partial gene deletions may be rescued by homologous recombination. Point mutations and frame-shift mutations can be reversed.**



Section III-E-1 Revised – cont. . .

- ❑ **For emerging and less characterized viruses, impairment of replication and transmission can be assured by a minimum 50% deletion of the viral genome.**



Section III-E-1 Proposal April 2010*

- **Recombinant nucleic acids from a eukaryotic virus (excluding *Variola major* and *V. minor*) and/or synthetic nucleic acids molecules based on a sequence from a eukaryotic virus (excluding *Variola major* and *V. minor*) may be propagated and maintained in cells in tissue culture using BL1 containment if:**

* Published in Federal Register on April 22, 2010 (75 FR 21008)



III-E-1 Proposed

- **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, or**
- **For Risk Group 3 or 4 viruses no more than half of the genome is present, (all viruses from a single Family being considered identical). The nucleic acids may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than one-half of a genome.**

* Published in Federal Register on April 22, 2010 (75 FR 21008)



III-E-1 Proposed

- In addition, there must be evidence that 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.**
- If a gene deletion is the basis for a reduction in containment, sequence or other appropriate data should be submitted to the IBC to demonstrate that the deleted function(s) cannot be rescued by homologous recombination.**

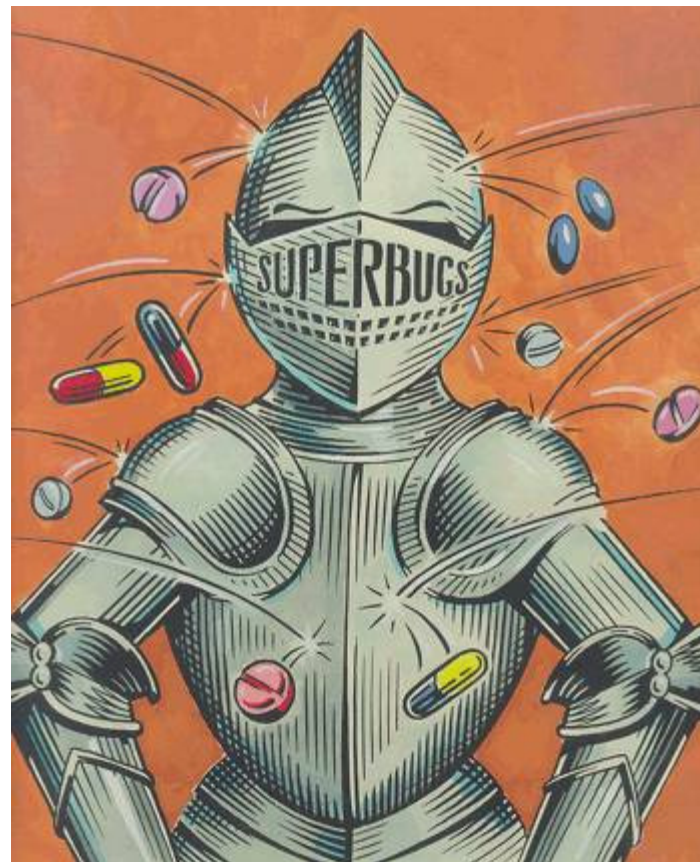


III-E-1 Proposed

- ❑ **It must be demonstrated that the cells lack helper virus for specific Families of defective viruses being used. If helper virus is present, Section III-D-3 applies and IBC review is required prior to initiation.**
- ❑ **A minimum of BL2 containment is required for experiments with retroviruses that have the potential to transduce human cells and cause insertional mutagenesis.**



Section III-A-1 Bugs and Drugs





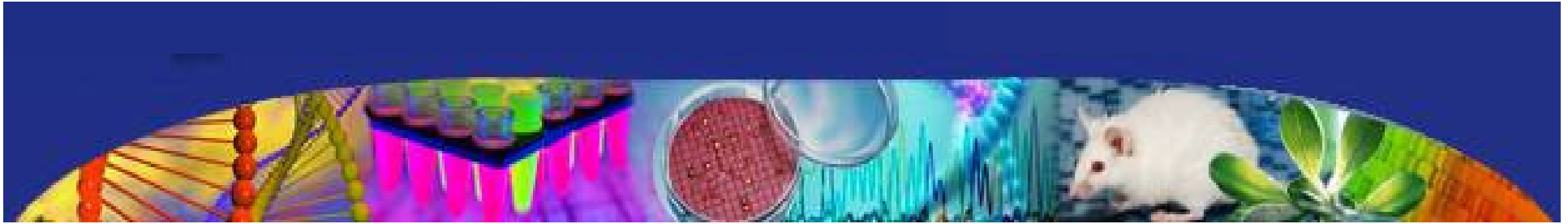
Section III-A-1- Major Actions

- 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.**



Public Health and Scientific Research

- ❑ The deliberate creation of a microorganism that may be more difficult to manage or treat creates a public health risk**
- ❑ This public health risk is not only of local concern and warrants a more thorough in-depth review, expert consultation and public discussion in the context of the RAC.**



Clinical Utility Assessment

- ❑ **Is this drug considered first or second line?**
 - **If the organism is made resistant to this drug how many alternative drugs would be available?**
 - **Is there the possibility of causing cross-resistance to other drugs in the same or different classes?**

- ❑ **Even if not first or second line, is this drug indicated in certain populations (e.g. pregnant women, children) or used as first line therapy in other countries?**



Should these Experiments be Reviewed?

- ❑ Ciprofloxacin resistance into *Neisseria meningitidis*
- ❑ Vancomycin resistance into *Staph. aureus*
- ❑ Ceftriaxone resistance into *Neisseria gonorrhoeae*
- ❑ Pyrimethamine resistance into *Toxoplasma gondii*



Proposed Language March 2009

- ❑ The deliberate transfer of a drug resistance trait to microorganisms
- ❑ **If that microorganism is not known to acquire the trait naturally, and**
- ❑ **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 March 2009

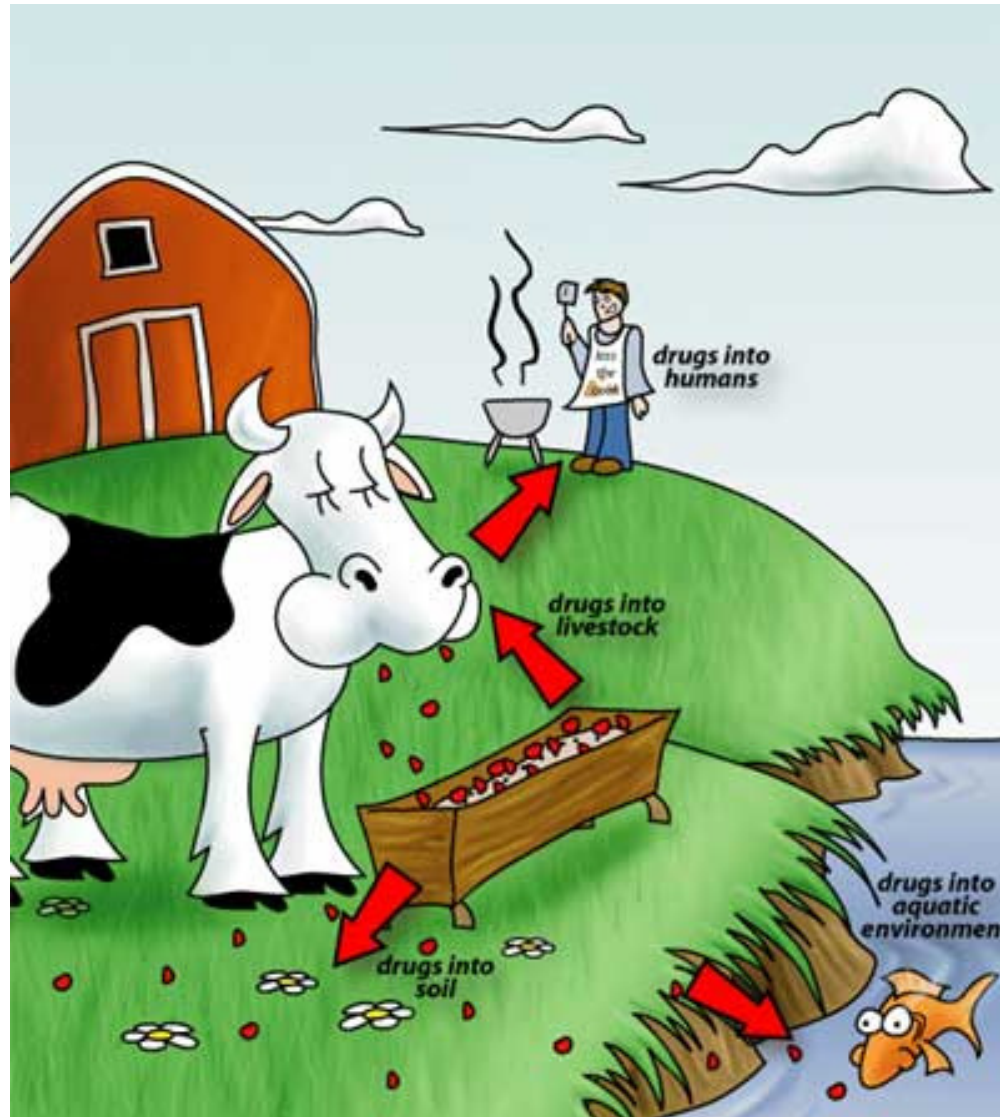
- ❑ **Even if an alternative drug or drugs exist for the control or management of disease, it is important to consider how the research might affect the ability to control infection in certain groups or subgroups by putting them at risk of developing an infection by such microorganism for which alternative 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.**



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.**

Research is not the Problem





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.**



Revised RAC Recommendation*

- ❑ **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 drugs 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.**

Discussed at December 2009 Meeting of the RAC



Revised RAC Recommendation*

- 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.**

Discussed at December 2009 Meeting of the RAC



Summary of RAC Recommendations

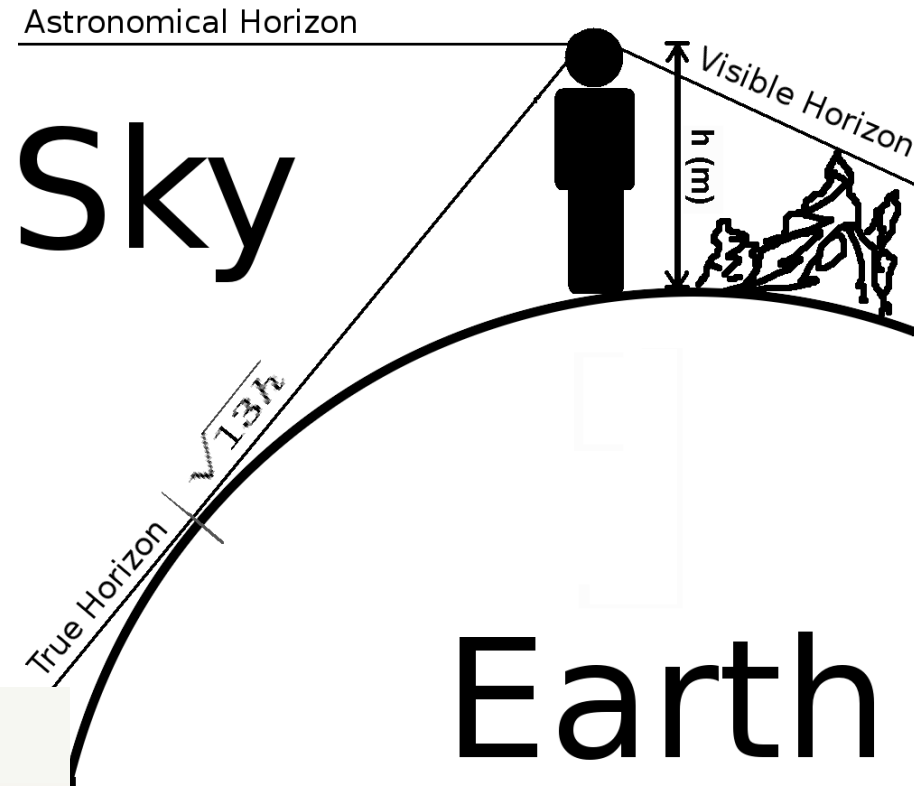
- ❑ **Scope of the *NIH Guidelines* should be expanded to specifically cover synthetic nucleic acids**
- ❑ **If the synthetic nucleic acid can not replicate in any cell, basic research will generally be exempt except for integrating molecules, or those that code for toxins**
- ❑ **Synthetic nucleic acids used in human gene transfer remains an outstanding question**



Summary of RAC Recommendations

- ❑ Section III-E-1 should have a quantitative and qualitative test**
- ❑ Section III-A-1 – Major Actions will not fundamentally change but clarification will be provided regarding consultation with OBA and/or the RAC**

ON THE HORIZON





Transgenic Mice

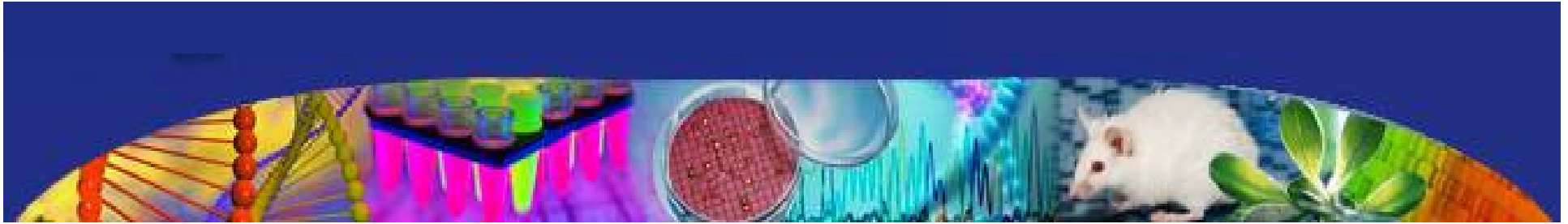
Section III-E-3

- ❑ Experiments that involve the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents) and require BL 1 containment may be initiated upon registration with the IBC
- ❑ “Generation” of a transgenic rodent includes mating between two different transgenic rodents or mating of a transgenic rodent and a non-transgenic rodent
 - Breeding of two identical transgenic rodents to maintain a line is not subject to this section of the *NIH Guidelines*



Transgenic Mice

- ❑ **Transgenic rodents that may be contained under BL1 conditions do not pose an appreciable biosafety risk to humans**
- ❑ **The *NIH Guidelines* currently exempts the purchase or transfer of transgenic rodents that require BL1 containment (Appendix C-VI)**



Transgenic Mice

- The overwhelming majority of matings of transgenic rodents that require BL1 containment will result in a rodent that can be housed at BL1 and would therefore not pose an appreciable risk to human health**
- While each registration is not a significant burden, the total number of registrations required leads to an administrative burden on the IBC and researchers that does not appear to be commensurate with the biosafety risk**



RAC Discussions

- Proposal discussed at RAC meeting in March 2010 to exempt most matings between BL1 transgenic mice except those with transgenes coding for a prion or amyloids, those containing more than 50 % of an exogenous viral genome and transgene under control of a retroviral LTR**
- Revised proposal to be reviewed at the June 16-17, 2010 meeting of the RAC**



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