MSC 7806, Bethesda, MD 20892, (301) 435–2037, *mackj2@csr.nih.gov.*

Name of Committee: Endocrinology, Metabolism, Nutrition and Reproductive Sciences, Integrated Review Group, Molecular and Cellular Endocrinology Study Section.

Date: October 9, 2012.

Time: 8 a.m. to 6 p.m.

Agenda: To review and evaluate grant applications.

Place: Embassy Suites at the Chevy Chase Pavilion, 4300 Military Road NW., Washington, DC 20015.

Contact Person: John Bleasdale, Ph.D., Scientific Review Officer, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 6170 MSC 7892, Bethesda, MD 20892, 301–435–4514, *bleasdaleje@csr.nih.gov.*

Name of Committee: Integrative, Functional and Cognitive Neuroscience Integrated Review Group, Auditory System Study Section.

Date: October 9, 2012.

Time: 8 a.m. to 6:30 p.m.

Agenda: To review and evaluate grant applications.

Place: Melrose Hotel, 2430 Pennsylvania Avenue NW., Washington, DC 20037.

Contact Person: Lynn E Luethke, Ph.D., Scientific Review Officer, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5166, MSC 7844, Bethesda, MD 20892, (301) 806–3323, *luethkel@csr.nih.gov.*

Name of Committee: Digestive, Kidney and Urological Systems Integrated Review Group, Pathobiology of Kidney Disease Study Section.

Date: October 9–10, 2012.

Time: 8 a.m. to 6 p.m.

Agenda: To review and evaluate grant applications.

Place: Hyatt Regency Bethesda, One Bethesda Metro Center, 7400 Wisconsin Avenue, Bethesda, MD 20814.

Contact Person: Atul Sahai, Ph.D., Scientific Review Officer, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 2188, MSC 7818, Bethesda, MD 20892, 301–435–1198, *sahaia@csr.nih.gov.*

Name of Committee: Center for Scientific Review Special Emphasis Panel, Member Conflict: Cell Biology.

Date: October 9, 2012.

Time: 11 a.m. to 1 p.m.

Agenda: To review and evaluate grant applications.

Place: National Institutes of Health, 6701 Rockledge Drive, Bethesda, MD 20892, (Telephone Conference Call).

Contact Person: Wallace Ip, Ph.D., Scientific Review Officer, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5128, MSC 7840, Bethesda, MD 20892, 301–435–1191, *ipws@mail.nih.gov.*

(Catalogue of Federal Domestic Assistance Program Nos. 93.306, Comparative Medicine; 93.333, Clinical Research, 93.306, 93.333, 93.337, 93.393–93.396, 93.837–93.844, 93.846–93.878, 93.892, 93.893, National Institutes of Health, HHS)

Dated: August 29, 2012.

David Clary,

Program Analyst, Office of Federal Advisory Committee Policy.

[FR Doc. 2012–21753 Filed 9–4–12; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institute of Biomedical Imaging and Bioengineering; Notice of Closed Meeting

Pursuant to section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. App.), notice is hereby given of the following meeting.

The meeting will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Institute of Biomedical Imaging and Bioengineering Special Emphasis Panel, ZEB1 OSR–D(J2) P Tissue Engineering Resource Center (P41).

Date: November 7–9, 2012.

Time: 6 p.m. to 12 p.m.

Agenda: To review and evaluate grant applications.

Place: Best Western Hotel III Tria, 220 Alewife Brook Parkway, Cambridge, MA 02138.

Contact Person: John K. Hayes, Ph.D., Scientific Review Officer, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, 6707 Democracy Boulevard, Room 959, Bethesda, MD 20892, 301–451–3398, *hayesj@mail.nih.gov*.

Dated: August 29, 2012.

David Clary,

Program Analyst, Office of Federal Advisory Committee Policy.

[FR Doc. 2012–21751 Filed 9–4–12; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Final Action Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

SUMMARY: On March 4, 2009, the National Institutes of Health (NIH) Office of Biotechnology Activities, Office of Science Policy (NIH/OBA) published a proposal in the **Federal Register** (74 FR 9411) to revise the *NIH Guidelines* in two regards. The first was to address biosafety considerations for research with synthetic nucleic acids. The proposal modified the scope of the *NIH Guidelines* specifically to cover certain basic and clinical research with nucleic acid molecules created solely by synthetic means. The second proposed revision was to modify the criteria for determining whether an experiment to introduce drug resistance into a microorganism must be reviewed by the Recombinant DNA Advisory Committee (RAC) and approved by the NIH Director (as a Major Action under Section III–A–1–a of the *NIH Guidelines*). Comments submitted were discussed at the ''NIH Public Consultation on Proposed Changes to the *NIH Guidelines* for Synthetic Nucleic Acids'' on June 23, 2009 (*http://oba.od.nih.gov/rdna_rac/rac_pub_con.html''.*

This notice sets forth final changes to the *NIH Guidelines* regarding those two proposals. The scope of the *NIH Guidelines* is being modified to cover certain classes of basic and clinical research with synthetic nucleic acids while exempting others. As discussed herein, the majority of research with synthetic nucleic acids that are not designed to replicate does not raise significant biosafety concerns that warrant oversight under the *NIH Guidelines*. Because of the modification of the scope of the *NIH Guidelines*, the title of the *NIH Guidelines* will be revised from *NIH Guidelines for Research Involving Recombinant DNA Molecules* to *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids Molecules.*

These changes also clarify the criteria for determining whether an experiment to introduce drug resistance into a microorganism raises sufficient public health issues to warrant the experiment being reviewed by the RAC and approved by the NIH Director under Section III–A–1–a of the *NIH Guidelines*. While the current criteria for determining whether an experiment requires review under Section III–A–1–a are being retained, additional language is being added regarding the assessment of whether a drug is therapeutically useful. In addition, NIH/OBA has clarified that Institutional Biosafety Committees (IBCs) can consult with NIH/OBA regarding a specific experiment that does not meet the criteria for review under Section III–A–1–a but nonetheless raises important public health issues. Finally, a section is added to give NIH/OBA the authority

to approve new experiments utilizing the same drug resistance trait and organism used in an experiment previously reviewed by the RAC and approved by the NIH Director.

In March 2009, NIH/OBA also proposed changes to Section III-E–1 of the *NIH Guidelines*, which sets containment for recombinant experiments involving two-thirds or less of the genome of certain viruses in tissue culture. In response to the comments on the proposed changes to Section III–E–1, NIH/OBA revised the proposal and published a notice for comment on April 22, 2010 (75 FR 21008). Comments received in response to this notice were discussed at the June 16, 2010, public meeting of the RAC and additional discussions of subsequent revisions to the proposed changes took place at the June 7, 2011, meeting of the RAC. As these changes are not yet finalized, NIH/OBA will move forward with the other changes outlined below pending finalization of changes to Section III–E–1.

DATES: These changes are effective March 5, 2013. All ongoing and proposed experiments that will be newly subject to these amended *NIH Guidelines* will need to be registered by the Principal Investigator with the IBC by the effective date listed above. The six-month time frame was deemed sufficient to allow institutions to develop new procedures, as well as outreach and training for investigators whose research will now be subject to the *NIH Guidelines*. While NIH/OBA does not anticipate a significant increase in experiments subject to the *NIH Guidelines,* it is important that institutions be afforded ample time to implement effectively these changes.

FOR FURTHER INFORMATION CONTACT: If you have questions, or require additional information about these proposed changes, please contact NIH/OBA by e-mail at *oba@od.nih.gov,* by telephone at 301–496–9838, by fax to 301–496–9839, or by mail to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, Maryland 20892.

SUPPLEMENTARY INFORMATION: As discussed in more detail in the March 2009 **Federal Register** notice, nucleic acid (NA) synthesis technology, in combination with other rapidly evolving capabilities in the life sciences, such as directed molecular evolution and viral reverse genetics, has the potential to accelerate scientific discovery, yield new therapeutics for disease, and facilitate the modification of existing organisms or the creation of new organisms, including pathogens.

The impetus for these changes to the *NIH Guidelines* is two-fold: (1) Recognition that appropriate biosafety containment of an agent is critical regardless of the technology used to generate that agent (i.e., recombinant DNA or synthetic biology), and (2) a recommendation from the National Science Advisory Board for Biosecurity (NSABB). The NSABB was formed to advise the U.S. Government on strategies for minimizing the potential for misuse of information, products, and technologies from life sciences research, taking into consideration both national security concerns and the needs of the research community. In 2006, the NSABB published a report titled ''Addressing Biosecurity Concerns Related to the Synthesis of Select Agents'' (available at *http://oba.od.nih.gov/biosecurity/pdf/Final_NSABB_Report_on_Synthetic_Genomics.pdf).*

In that report, the NSABB noted that practitioners of synthetic genomics or researchers using synthetic nucleic acids in the emerging field of synthetic biology are not necessarily biologists and, therefore, may not have been trained in biosafety. These researchers may be uncertain about how to conduct a risk assessment, as required for research currently subject to the *NIH Guidelines*, and when to have their work undergo review by an IBC. The NSABB report recommended that the U.S. Government ''examine the language and implementation of current biosafety guidance to ensure that such guidelines and regulations provide adequate guidance for working with synthetically derived DNA and are understood by all those working in areas addressed by the guidelines.''

The recommendation on the need for examination of existing biosafety guidance was accepted by the U.S. Government with the understanding that implementation would be through examination and modification of the *NIH Guidelines*, as appropriate. The changes to the *NIH Guidelines* would then be cross-referenced in the joint publication by the U.S. Centers for Disease Control and Prevention and NIH titled: *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) (available at *http://www.cdc.gov/biosafety/publications/bmbl5/index.htm*).

As stated in the March 2009 **Federal Register** notice, these changes were developed in consultation with the RAC. A total of 50 comments were received in response to the March 2009 **Federal Register** notice from individuals, academic and government researchers, private pharmaceutical companies and trade organizations that represent the biosafety community, researchers in gene and cell therapy, and microbiologists. In addition, a day-long public discussion of the proposed changes was held on June 23, 2009, in Arlington, Virginia. The agenda and webcast of that meeting are available at the following URL: *http://oba.od.nih.gov/rdna_rac/rac_pub_con.html.*

The *NIH Guidelines* currently apply to all recombinant DNA research that is conducted at or sponsored by institutions that receive NIH funding for any research involving recombinant DNA. In addition, some federal agencies, including the U.S. Departments of Energy, Veterans Affairs, and Agriculture, currently have policies in place stating that all recombinant DNA research conducted by or funded by these agencies must comply with the *NIH Guidelines*. While the *NIH Guidelines* may not apply to all Government-funded and privately funded research, it may be used as a tool for the entire research community to understand the potential biosafety implications of this type of research.

Summary of Comments

All of the comments submitted in response to the **Federal Register** notice are available for review on the NIH/OBA Web site at: *http://oba.od.nih.gov/rdna_rac/rac_pub_con.html.* The public comments generally fell into two groups: (1) Comments on the proposed changes regarding research with synthetic nucleic acids and (2) comments on the proposed changes to Section III-A–1-a (experiments involving the deliberate transfer of a drug resistance trait into microorganisms). Overall, the comments favored modifying the scope of the *NIH Guidelines* to include research with synthetic nucleic acids. As one commenter noted, ''With the ability to chemically synthesize entire genes or substantial portions of viral genomes, such synthetic entities would have the potential to (1) Express proteins, (2) replicate in cells, and (3) integrate into the host genome. As such, these entities warrant the same scrutiny as traditional recombinant DNA with respect to studies being conducted in [a] research laboratory and when being considered for use in human subjects, and thus should be subject to NIH/OBA registration and RAC review.'' However, there were concerns that the proposed amendments would lead to oversight of the synthesis of small nucleic acid primers used in basic research. This was a misinterpretation of the proposed

changes; research with nucleic acids that are not in cells or organisms is not subject to the *NIH Guidelines* and the proposed exemption for non-replicating synthetic nucleic acids, discussed herein, would also preclude these constructs from being subject to the *NIH Guidelines*.

Most of the comments regarding synthetic nucleic acids and the *NIH Guidelines* focused on whether certain synthetic nucleic acids used in human clinical trials should also be exempt from the *NIH Guidelines* and in particular from the requirements for submission and review of human gene transfer trials (as outlined in Appendix M of the *NIH Guidelines*). These comments directly addressed a question posed in the March 2009 **Federal Register**: ''For human gene transfer research, are there classes of non-replicating, synthetic molecules that should be exempt due to lower potential risk (*e.g.* antisense RNA, RNAi)? If so, what criteria should be applied to determine such classes?''

Many of the respondents to this question were involved in developing such products to be used as therapeutics or represent companies and investigators involved in such research. As discussed in more detail herein, the respondents argued that small non-replicating synthetic nucleic acids used as therapeutics are more akin to small molecule drugs than traditional gene transfer agents. A session at the June 23, 2009, public consultation focused on whether certain non-replicating synthetic nucleic acids used in human clinical trials should be exempted from the *NIH Guidelines* due to characteristics that are distinct from recombinant molecules as currently defined in the *NIH Guidelines.*

The second set of comments focused on the proposed changes to Section III–A–1–a, which addresses certain experiments that involve the introduction of drug resistance into microorganisms. The comments uniformly disagreed with the proposed changes stating that the new proposed criteria were too broad and would lead to federal review of experiments that did not raise public health issues warranting heightened scrutiny. Moreover, they stated that there is no evidence that the current language had failed to serve the public health and therefore the changes were not warranted given the potential problems raised by expanding such review. As discussed herein, the III–A–1–a language in the current *NIH Guidelines* (October 2011 version) will be retained.

The following paragraphs review (1) The specific comments received on each section of the *NIH Guidelines,* both the written comments and those received at public meeting; (2) NIH/OBA's response to those comments; and (3) the final changes to the *NIH Guidelines.*

Amendments to the NIH Guidelines

In order to ensure that biosafety considerations of synthetic biology research are addressed appropriately, changes are being made to the following sections of the *NIH Guidelines:*
the NIH Guidelines

Section I. Scope of the NIH Guidelines
Section I–B. Definition of Recombinant DNA
Section I–C. General Applicability
Section III–C. Experiments Involving the Deliberate Transfer of Recombinant DNA,or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants
Section III–F. Exempt Experiments
Section IV–A. Policy
Section II–A–3. Comprehensive Risk Assessment

As discussed herein, the *NIH Guidelines* will no longer be limited to oversight of research with recombinant nucleic acid molecules but will also address research with certain synthetic nucleic acids. Throughout the *NIH Guidelines,* the term ''recombinant DNA molecules'' will be replaced with ''recombinant or synthetic nucleic acids,'' which will encompass research with either recombinant or synthetic or both types of nucleic acids. This change will not be made to the name of the Recombinant DNA Advisory Committee, although the Committee will provide advice on both recombinant and synthetic nucleic acid research.

In addition to the changes being made specifically to address research with synthetic nucleic acids, the following sections are also being revised:

Section III–A–1. Major Actions under the NIH Guidelines
Section III–B. Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval before Initiation

Title of the NIH Guidelines

The title of the document will be changed from the *NIH Guidelines for Research Involving Recombinant DNA Molecules* to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.* NIH received no comments regarding the proposed change to the title of the *NIH Guidelines.*

Section I. Scope of the NIH Guidelines

To clarify the applicability of the *NIH Guidelines* to research involving synthetic nucleic acids, modifications were proposed to Section I, Scope of the *NIH Guidelines.* Section I–A (Purpose) of the *NIH Guidelines* previously stated:

> The purpose of the *NIH Guidelines* is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I–A was proposed to be changed to:

> 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 wholly or partially containing functional equivalents of nucleotides, and (iii) organisms and viruses containing such molecules.

NIH/OBA received one comment regarding the use of the term ''constructing'' in reference to synthetic nucleic acids. The concern was that the *NIH Guidelines* would govern the chemical synthesis of nucleic acids. However, this language was not a revision to the original scope of the *NIH Guidelines.* While the scope of the *NIH Guidelines* has always referred to ''constructing'' or construction of recombinant nucleic acids, the *NIH Guidelines* then exempts research with nucleic acids that are not contained in cells, organisms, or viruses. Therefore, the chemical synthesis of nucleic acids not placed in cells, organisms, or viruses would likewise be exempt; the *NIH Guidelines* will only apply once synthetic nucleic acids are placed in a biological system.

NIH/OBA also received comments requesting a definition of the term ''functional equivalents of nucleotides.'' This term was intended to capture synthetic nucleic acids that contain nucleotides that have been chemically modified and do not have the same chemical structure as the nucleotides in naturally occurring nucleic acids (see, for example, S. Benner, Redesigning Genetics. *Science.* 306, 625–626 (2004)). For clarity, the term ''functional equivalents'' has been changed to ''nucleotides that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules.''

Thus, the amended Section 1–A Purpose will state:

> Section 1–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 with naturally occurring nucleic acid molecules, and (iii) cells, organisms, and viruses containing such molecules.

As a result of these modifications, the *NIH Guidelines* will apply (unless otherwise exempted by other sections of the *NIH Guidelines, e.g.* III–F) to both recombinant and synthetically derived nucleic acids, including those that are chemically or otherwise modified analogs of nucleotides (*e.g.* , morpholinos).

Section I–B. Definition of Recombinant Nucleic Acids

The current definition of a recombinant DNA molecule in the *NIH Guidelines* (Section I–B) only explicitly refers to DNA and requires that segments be joined, which may not need to occur in research with synthetic nucleic acids. The revision to this section largely retains the definition of recombinant DNA but also adds a definition for synthetic nucleic acids that are created without joining segments of nucleic acids.

Section I–B also contains a paragraph that states:

> Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (*e.g.* , a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines.*

A second paragraph in the definition states:

> Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

The final changes eliminate the first paragraph above, referring to synthetic DNA segments, because the *NIH Guidelines* now specifically includes an exemption for certain low-risk synthetic constructs (see III–F–1). For consistency, the second paragraph on transposons was moved to the portion of the *NIH Guidelines* that covers exemptions (Section III–F). The NIH received no comments on eliminating the first paragraph and moving the second paragraph; therefore these changes are being implemented.

With respect to the definition of recombinant and synthetic nucleic acids, NIH/OBA received several comments with suggestions to use a single definition for recombinant and synthetic nucleic acids. NIH/OBA considered these proposals carefully but decided instead to largely retain the original definition of recombinant DNA, with clarification that it applies to both DNA and RNA and to add a new definition of synthetic nucleic acids. This was done because the definition of recombinant DNA will not change with this revision to the *NIH Guidelines.* As in the Scope section, the modification to the language ''functional equivalent'' will be included in the definition as well.

Section I–B is changed as follows:

> Section I–B. Definition of Recombinant and Synthetic Nucleic Acid Molecules:
>
> In the context of the *NIH Guidelines,* recombinant and synthetic nucleic acids are defined as:
>
> (i) molecules that a) are constructed by joining nucleic acid molecules and b) can replicate in a living cell, *i.e.* , recombinant nucleic acids;
>
> (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.

Section I–C. General Applicability

In the March 2009 **Federal Register** notice, NIH/OBA stated that it would change, throughout the *NIH Guidelines,* as appropriate, the term ''recombinant DNA molecules'' to ''recombinant and synthetic nucleic acid molecules.'' NIH/OBA received a comment that this substitution would imply that the *NIH Guidelines* only apply to research that uses synthetic and recombinant nucleic acids together, not just recombinant nucleic acid molecules or synthetic nucleic acid molecules alone. NIH/OBA agrees with the comment on the original proposed language and instead will replace, where appropriate recombinant DNA with ''recombinant or synthetic nucleic acid molecules'' to specify that the section applies to research with recombinant or synthetic nucleic acids or both. Section 1–C–1 currently states:

> **Section I–C. General Applicability**
>
> Section I–C–1. The *NIH Guidelines* are applicable to:
>
> Section I–C–1–a. All recombinant DNA research within the United States (U.S.) or its territories that is within the category of research described in either Section I–C–1–a–(1) or Section I–C–1–a–(2).
>
> Section I–C–1–a–(1). Research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines.*
>
> Section I–C–1–a–(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.
>
> Section I–C–1–b. All recombinant DNA research performed abroad that is within the category of research described in either Section I–C–1–b–(1) or Section I–C–1–b–(2).
>
> Section I–C–1–b–(1). Research supported by NIH funds.
>
> Section I–C–1–b–(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I–C will now read:

> **Section I–C. General Applicability**
>
> Section I–C–1. The *NIH Guidelines* are applicable to:
>
> Section I–C–1–a. All recombinant or synthetic nucleic acid research within the United States (U.S.) or its territories that is within the category of research described in either Section I–C–1–a–(1) or Section I–C–1–a–(2).
>
> Section I–C–1–a–(1). Research that is conducted at or sponsored by an institution that receives any support for recombinant or synthetic nucleic acid research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant or synthetic nucleic acids must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines.*
>
> Section I–C–1–a–(2). Research that involves testing in humans of materials containing recombinant or synthetic nucleic acids developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.
>
> Section I–C–1–b. All recombinant or synthetic nucleic acid research performed abroad that is within the category of research described in either Section I–C–1–b–(1) or Section I–C–1–b–(2).
>
> Section I–C–1–b–(1). Research supported by NIH funds.
>
> Section I–C–1–b–(2). Research that involves testing in humans of materials containing recombinant or synthetic nucleic acids developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section III–C–1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived From Recombinant DNA, Into One or More Human Research Participants

In March 2009, NIH/OBA proposed the following change to the definition of human gene transfer:

For an experiment involving the deliberate transfer of recombinant and/or synthetic nucleic acids into one or more human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in Section I–E–7) until the RAC review process has been completed (see Appendix M–I–B, RAC Review Requirements).

NIH/OBA had proposed exempting from the *NIH Guidelines* non-clinical research with certain synthetic nucleic acids but did not propose to extend that exemption to the use of these constructs in a clinical setting. NIH/OBA noted that many gene transfer trials that are currently subject to the *NIH Guidelines* use non-replicating recombinant molecules because they are derived through recombinant technology which involves replication. NIH/OBA proposed that there are shared safety issues raised by clinical protocols that use synthetic non-replicating nucleic acids and those that use non-replicating recombinant vectors.

The proposal to exempt basic research with non-replicating synthetic nucleic acids but not to extend that exemption to human gene transfer research was based on the differences in the potential health risk from inadvertent exposure during basic or preclinical work versus intentional exposure in a clinical setting. The doses and routes of administration used in human gene transfer generally increase the safety risks as compared to exposures that may occur in a basic research setting. Moreover, the clinical safety risks to be considered for human gene transfer are not limited to the replicative nature of the vector but include transgene effects, risks of insertional mutagenesis, immunological responses, and potential epigenetic changes. Human gene transfer also raises scientific, medical, social, and ethical considerations that warrant special attention and public discussion.

NIH/OBA received a number of comments from industry, including several comments from the Oligonucleotide Safety Working Group (OSWG), which represents 70 pharmaceutical and regulatory professionals involved in the clinical development of oligonucleotide-based therapies. The OSWG stated that synthetic nucleic acid oligonucleotides that are less than 100 nucleotides and are not delivered in a bacterial or viral vector are more analogous to small molecule drugs than to the agents currently used in human gene transfer. They noted that these constructs can be distinguished from the recombinant agents currently used in human gene transfer by their inability to integrate into the genome or replicate in cells, their lack of a transgene that can be transcribed into RNA or translated into a protein, and their transient nature, i.e., they are degraded within days. They recognized that the review of gene transfer protocols by the RAC is useful to address such risks in gene transfer, but they did not believe that review should be extended to these constructs merely because they are synthetic nucleic acids. They noted that no significant safety issues have arisen in the ongoing Phase I and Phase II clinical trials using short-interfering RNA oligonucleotides (siRNAs). In addition to these trials, there is significant interest in developing clinical applications directed at microRNAs (miRNAs). For recent reviews of the field see K. Tiemann, J. Rossi, RNAi-based therapeutics-current status, challenges and prospects. *EMBO Mol. Med.* 1,142–151 (2009), and D. Grimm, M. A. Kay, Therapeutic application of RNAi: is mRNA targeting finally ready for prime time. *The Journal of Clinical Investigation.* 117(12), 3633–3641 (2007).

While this clinical data is reassuring, several preclinical investigations raised important questions regarding the current understanding about the mechanisms underlying the clinical action of these constructs. For example, clinical trials using a siRNA against vascular endothelial growth factor-A (VEGFA) or its receptor (VEGFR1) in patients with blinding choroidal neovascularization (CNV) from age-related macular degeneration have demonstrated promising results. The hypothesis is that the siRNAs that are specific for VEGFA or its receptor are responsible for the clinical responses seen. In 2008, M.E. Kleinman, *et al.* found that a siRNA that did not specifically target VEGFA or VEGFR1 could also suppress CNV in mice through an immune response generated through toll-like receptors and induction of interferon-gamma and interleukin-12 (see M.E., Kleinman, *et al.*, Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. *Nature.* 452, 591–598 (2008)). In another study, investigators developed anti-macrophage inhibitory factor (MIF) siRNAs designed to block MIF expression in mammary adenocarcinoma cells (MCF–7). MIF is a ''pleiotropic cytokine with well described roles in cell proliferation, tumorigenesis and angiogenesis'' (M.E. Armstrong, *et al.* , Small Interfering RNAs Induce Macrophage Migration Inhibitory Factor Production and Proliferation in Breast Cancer Cells via a Double Stranded RNA-Dependent Protein Kinase-Dependent Mechanism. *J. Imm.*180, 7125–7133 (2008)). MIF has been shown to exert its actions through activation of CD44 and enhanced CD44 activation has been shown to promote breast cancer cell invasion. Unexpectedly, when these anti-MIF siRNAs were delivered to MCF–7 cells, the result was increased MIF production and an increase in proliferation of these cells.

In addition to questions regarding the mechanisms of action and potential off target effects raised by these publications, the RAC discussed whether administration of these synthetic RNAs could potentially lead to long-term gene silencing and phenotypic changes. As stated by the OSWG in their comments, one of the reasons for the RAC oversight of recombinant research is to assess the potential for alteration of a research participant's DNA, which could have unknown and unintended consequences. Recent research indicates that siRNA and miRNAs may be involved in long-term gene silencing (A. Verdel, *et al.*, Common themes in siRNA-mediated epigenetic silencing pathways. *Int. J. Dev. Biol.* 53, 245–257 (2009); D. H. Kim, *et al.* , MicroRNA-directed transcriptional gene silencing in mammalian cells. *PNAS.* 105(42), 16230–16235 (2008)). The implications of these preliminary findings and whether such effects on genes are fundamentally different than those exerted by certain small molecules, for example histone deacetylation inhibitors, remains an open question: It has been shown that histone deacetylation can silence genes through chromatin modification and deacetylation of the chromatin histone protein. Histone deacetylase inhibitors are in development as potential cancer therapeutics (see *e.g.* , A.A Lane, B.A. Chabner, Histone deacetylase inhibitors in cancer therapy. *J. Clin. Oncol.* 27(32), 5459–68 (2009)).

After considering the comments by the OSWG and other interested stakeholders, as well as the available literature, the RAC initially recommended that NIH/OBA consider an exemption for certain well characterized synthetic oligonucleotides, such as synthetic DNA

oligonucleotides that have been in clinical development for a number of years and whose mechanism of action is well understood. The RAC had reservations regarding extending that exemption to all synthetic RNA oligonucleotides because of the emerging literature that raised questions regarding our understanding of the potentially complex biological pathways being targeted. Indeed certain pathways are highly conserved across species and individual miRNAs have been shown to suppress the production of hundreds of proteins (D. Baek, *et al.* The impact of microRNAs on protein output. *Nature.* 455, 64–71(2008)). Additionally, the RAC considered that review of clinical protocols that administered RNA oligonucleotides without a vector would inform and enhance the review of similar protocols that use vectors (*e.g.* , short hairpin RNA (shRNA) expressed from a plasmid) and also inform the field and promote the exchange of data that could enhance its development. The RAC noted that this review might only be for several years until more data were developed.

The RAC, however, continued to reflect upon the data and considered additional stakeholder input. Further discussions were held with leading experts on RNAi, including Noble Prize laureates Dr. Phillip Sharp and Dr. Craig Mello. The RAC carefully considered the differences between synthetic nucleic acids that are not delivered in vectors and those delivered in bacterial or viral vectors, taking into account their inability to replicate, integrate, or be transcribed or translated. Finally, given the uncertain significance of preclinical data in the absence of adverse effects in the ongoing clinical trials, the RAC concluded that oversight is not warranted at this time. NIH/OBA concurs with this assessment, and the *NIH Guidelines* will only apply to recombinant constructs that are currently covered by the *NIH Guidelines* and those synthetic constructs that are equivalent to their recombinant counterparts, i.e. synthetic investigational agents that share the same characteristics as recombinant gene transfer constructs. However, in light of some unresolved outstanding questions regarding the mechanisms of actions of synthetic nucleic acids used clinically, including the potential for epigenetic changes, the RAC recommended NIH/OBA convene a meeting to further explore these questions. NIH/OBA hosted this meeting on December 15–16, 2011. (The agenda and slide presentations are available at: *http://oba.od.nih.gov/rdna/rdna_symposia.html.*)

Therefore, Section III–C–1 will be revised as follows:

Section III–C–1. Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or

2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:

a. Contain more than 100 nucleotides; or

b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or

c. Have the potential to replicate in a cell; or

d. Can be translated or transcribed.

No research participant shall be enrolled (see definition of enrollment in Section 1–E–7) until the RAC review process has been completed (see Appendix M–1–B, *RAC Review Requirements*).

Section III–F. Exempt Experiments

Modifications were proposed to augment or clarify experiments that are exempt from the *NIH Guidelines* (III–F). Certain nucleic acid molecules are exempt from the *NIH Guidelines* under Section III–F because (1) their introduction into a biological system is not expected to present a biosafety risk that requires review by an IBC, or (2) the introduction of these nucleic acid molecules into biological systems would be akin to processes of nucleic acid transfer that already occur in nature, so that the appropriate biosafety practices would be the same as those used for the natural organism and/or would be covered by other guidances.

As stated in the March 2009 **Federal Register** notice, with the exception of the new proposed Section III–F–1 discussed below, the exemptions from the current *NIH Guidelines* (October 2011) have been preserved with minor modifications. The addition of research with synthetic nucleic acids to the *NIH Guidelines* does not warrant modification of most of these exemptions except to extend them to synthetic constructs.

To emphasize that research exempt from the *NIH Guidelines* may still have biosafety considerations and that other standards of biosafety may apply, a modification is being made to the introductory language for this section. Section III–F currently states:

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required.

This portion is amended to read:

The following recombinant or synthetic nucleic acid molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required; however, other federal and state standards of biosafety may still apply to such research (for example, the Centers for Disease Control and Prevention (CDC)/NIH publication Biosafety in Microbiological and Biomedical Laboratories).

Section III–F–1. Exempt Experiments

A new entry under Section III–F was proposed to exempt from the *NIH Guidelines* synthetic nucleic acids that cannot replicate unless they are administered to one or more human research participant(s) (see Section III–C–1). This exemption was proposed so that the *NIH Guidelines* apply to synthetic nucleic acid research in a manner consistent with the current oversight of basic and preclinical recombinant DNA research. Currently oversight is limited to recombinant molecules that replicate or are derived from such molecules. The added section exempts basic, non-clinical research with synthetic nucleic acids that cannot replicate or are not derived from molecules that can replicate. The biosafety risks of using such constructs in basic and preclinical research are likely low. If a nucleic acid is incapable of replicating in a cell, any toxicity associated with that nucleic acid should be confined to that particular cell or organism, and spread to neighboring cells or organisms should not occur to any appreciable degree. This type of risk is analogous to that observed with chemical exposures, although nucleic acids are generally far less toxic than most chemicals.

NIH/OBA received a number of comments on this proposed exemption. Most of the comments questioned whether this exemption should be extended to certain non-replicating nucleic acids used in human gene transfer because such constructs are likely to pose quantitatively different risks than vector-based gene transfer. The response to these comments is articulated in the prior section of this notice that focuses on Section III–C–1.

With respect to basic research, NIH/OBA received comments questioning whether all non-replicating synthetic nucleic acids used in basic research pose sufficiently low biosafety risks to be exempt from the *NIH Guidelines.* Concerns were also raised about the use of synthetic non-replicating, integrating

viral vectors, such as lentiviral vectors, which could result in persistent transgene expression and have the potential to induce insertional oncogenesis. Non-replicating synthetic cassettes for toxins were also identified as raising potential biosafety risks as were oncogenes. In addition, clarification was sought regarding what was meant by the term ''replication.'' For example, would the following be considered replicating nucleic acids: (1) Plasmids lacking sequences to replicate in eukaryotic cells or (2) complementary DNAs (cDNAs) of positive strand RNA viruses, in which cDNA is not replicated but is transcribed into viral RNAs? In addition, another commenter asked why the exemption was limited to synthetic nucleic acids rather than all nucleic acids.

NIH/OBA carefully considered all of these comments. With respect to making this exemption apply generally to all nucleic acid constructs, recombinant and synthetic, NIH/OBA notes that the definition of recombinant DNA molecules, which remains unchanged, only includes molecules that can replicate in a living cell or molecules that result from the replication of those described above. Therefore, to include them in the exemption under III–F–1 would be redundant, as this exemption only applies to nucleic acids that cannot replicate and are not derived from those that can replicate. NIH/OBA acknowledges that research with an integrating vector could raise biosafety considerations even if the vector does not replicate. With respect to toxins, a non-replicating expression cassette can only express the toxin in a single cell and the toxin cannot spread from cell to cell, thereby limiting its toxic effect. Nonetheless, NIH/OBA agrees that constructs expressing toxins that are currently reviewed under Section III–B–1, Experiments Involving the Cloning of Toxin Molecules with LD50 of Less Than 100 Nanograms per Kilogram Body Weight, should remain subject to the NIH Guidelines. Indeed, under the current NIH Guidelines, even if an experiment falls under a Section III–F exemption, it may still be subject to review under Section III–B–1. For clarity, NIH/OBA therefore decided to specify that toxin-producing expression cassettes that would fall under Section III–B–1 will not be exempt under III–F.

Synthetic constructs that have the potential to integrate will not likewise be exempted because they could inadvertently activate an oncogene, or an integrating sequence containing an oncogene could inadvertently be integrated into a cell and persist and transform that cell and its progeny.

In the March 2009 **Federal Register** notice, Section III–F–1 was written so as to exempt from the NIH Guidelines ''Synthetic nucleic acids that cannot replicate, and that are not deliberately transferred into one or more human research participants (Section III–C and Appendix M).'' To clarify the interpretation of ''replicating,'' the language has been changed to match more closely that of the definition of recombinant DNA, ''cannot replicate in a living cell.'' This change is to make it clear that it is the ability to replicate in any cell type that determines whether the research is subject to the NIH Guidelines (*i.e.* , plasmids that can replicate in bacteria would be subject to the NIH Guidelines even if in eukaryotic cells). To address the cDNA of positive strand RNA viruses, the language has been changed to ''cannot replicate or generate nucleic acids that can replicate in a living cell.'' In addition, to make it clear that a synthetic replication incompetent virus is not exempt under this section of the NIH Guidelines, a parenthetical has been added to clarify that this section is meant to exempt only research with small synthetic oligonucleotides and expression cassettes, not synthetic viruses or bacteria that cannot replicate because of omission of one or more genes.

Section III–F–1 is changed to exempt the following experiments:

Section III–F–1. Those synthetic nucleic acids that: (1) Can neither replicate nor generate nucleic acids that can replicate in any living cell (*e.g.* , oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III–C, it is not exempt under this Section.

Section III–F–2. Exempt Experiments

Section III–F–1 will now be renumbered to III–F–2 and is amended to clarify that replicating nucleic acids that are not in cells, organisms, or viruses are exempt. The current NIH Guidelines only mentions organisms and viruses, and for clarity the term ''cells'' has been added. In addition, if a molecule is modified to facilitate entry into a cell, this will also not be exempt. Nucleic acids that are not in a biological system that will permit replication and that have not been modified to enable improved penetration of cell membranes are unlikely to have associated biosafety risks. NIH/OBA received no comments on this change.

The current Section III–F–1 states: ''Those that are not in organisms or viruses.''

Section III–F–1 is re-numbered to III–F–2 and will exempt the following experiments:

Section III–F–2. Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (*e.g.*, encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.

Sections III–F–3 through III–F–7

Revised Sections III–F–3 through III–F–7 retain exemptions that were in the current version of NIH Guidelines (October 2011) with minor revisions. There were no comments to the minor changes made in Sections III–F–3 through III–F–7. The following changes will be made for these Section III–F exemptions.

Section III–F–3. Exempt Experiments

Section III–F–2 exempts nucleic acid sequences that are essentially copies of those found in nature. The language has been modified as discussed in the March 2009 **Federal Register** notice by limiting this exemption to those nucleic acid sequences that exist contemporaneously in nature. Research in the lab with nucleic acid sequences for organisms that do not currently exist in nature, for example, an identical copy of the 1918 H1N1 influenza virus would not be exempt.

Section III–F–2 will be re-numbered to III–F–3 and will exempt the following experiments:

Section III–F–3. Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

Section III–F–4. Exempt Experiments

The current Section III–F–3 exempts nucleic acids that are being propagated only in a prokaryotic host that is either the natural host or a closely related strain of the natural host. Again such constructs may already exist outside of a laboratory. It is renumbered to Section III–F–4 and no amendment to the language is made. It exempts the following experiments:

Section III–F–4. Those that consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III–F–5: Exempt Experiments

The current Section III–F–4 exempts nucleic acids that are being propagated

in a eukaryotic host that is either the natural host or closely related strain of the natural host. Section III–F–4 is renumbered to Section III–F–5 and no amendment to the language is made. The following experiments are exempt per this section.

Section III–F–5. Those that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III–F–6. Exempt Experiments

Research that falls under Section III–F–6 (formerly Section III–F–5) is exempt because the manipulation of these nucleic acids in a laboratory setting would be equivalent to processes that occur in nature when certain organisms exchange genetic material via physiological processes (*e.g.* , bacterial conjugation). It is limited to those organisms, as specified in Appendices A–I through A–VI, that are already known to exchange DNA in nature. The current Section III–F–5 is renumbered to Section III–F–6 and no amendment to the language is made. The following experiments are exempt per this section.

Section III–F–6. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C–1–b–(1)–(c), Major Actions). See Appendices A–I through A–VI, Exemptions under Section III–F–6—Sublists of Natural Exchangers, for a list of natural exchangers that are exempt from the *NIH Guidelines*.

Additionally, Appendix A will be amended to reference Section III–F–6 rather than III–F–5.

Section III–F–7. Exempt Experiments

Research that falls under the proposed Section III–F–7 exemption also involves a natural physiological process, *i.e.* transposition. Transposons are nucleic acid molecules that exist in a wide variety of organisms from bacteria to humans. These molecules have the ability to move from one portion of an organism's genome to another. This new Section of III–F captures what was previously an exemption to the definition of a recombinant DNA molecule in the *NIH Guidelines* (Section I–B). Unless a transposon has been modified to be a recombinant molecule, genomic DNA that has acquired a transposon is not subject to the *NIH Guidelines*. Transposons that have not been modified by the insertion of recombinant or synthetic DNA are equivalent to what exists in nature and the process occurs naturally outside of a laboratory setting. The language from the definition of recombinant DNA (Section I–B) is being moved to this Section so that the definition of recombinant and synthetic nucleic acids found in Section I–B is solely a definition and does not include exemptions. The exemption described in Section I–B previously stated, ''Genomic DNA molecules of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.'' The exemption language has been simplified to make it clear that unmodified transposons used in research are not subject to the NIH Guidelines even if derived from a recombinant or synthetic system. In addition, the reference to only plants and bacteria has been removed since it is now known that transposons are also found in animals. Section III–F–7 will exempt the following experiments:

Section III–F–7. Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

Section III–F–8. Exempt Experiments

The current Section III–F–6 provides a mechanism by which other experiments that do not raise significant biosafety risks can be exempted from the NIH Guidelines after review by the RAC and approval by the NIH Director. The language has not been amended but, due to the insertion of two additional exemptions, it is being renumbered to Section III–F–8 and will exempt the following experiments:

Section III–F–8. Those that do not present a significant risk to health or the environment (see Section IV–C–1–b–(1)–(c), Major Actions), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, Exemptions under Section III–F–8 for other classes of experiments which are exempt from the *NIH Guidelines*.

Additionally, Appendix C will be amended to reference Section III–F–8 rather than III–F–6.

Section IV–A. Policy

Section IV–A addresses the roles and responsibilities of local institutions and investigators in implementing the *NIH Guidelines*. It contains a general policy statement that acknowledges the inability of the *NIH Guidelines* to address specifically all conceivable research or emerging techniques and therefore states that researchers and institutions should adhere to ''the intent of the *NIH Guidelines* as well as to their specifics.'' NIH/OBA received no comments on the proposed changes, which emphasize that the *NIH Guidelines* are expected to be modified to address new developments in research or scientific techniques. In addition, in rewriting this section of the *NIH Guidelines,* NIH/OBA has removed the sentence ''[G]eneral recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level,'' since the previous sentences adequately explains that the institution is accountable for implementation of the *NIH Guidelines*. Section IV–A currently states:

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The *NIH Guidelines* cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The *NIH Guidelines* are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. The *NIH Guidelines* will never be complete or final since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the institution and those associated with it to adhere to the intent of the *NIH Guidelines* as well as to their specifics. Each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all recombinant DNA research conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines*. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

Section IV–A is amended to read:

The safe conduct of experiments involving recombinant or synthetic nucleic acid molecules depends on the individual conducting such activities. The *NIH Guidelines* cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The *NIH Guidelines* are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. The *NIH Guidelines* will never be complete or final since all experiments

involving recombinant or synthetic nucleic acid molecules cannot be foreseen. The utilization of new genetic manipulation techniques may enable work previously conducted using recombinant means to be accomplished faster, more efficiently, or at larger scale. These techniques have not yet yielded organisms that present safety concerns that fall outside the current risk assessment framework used for recombinant nucleic acid research. Nonetheless, an appropriate risk assessment of experiments involving these techniques must be conducted taking into account the way these approaches may alter the risk assessment. As new techniques develop, the *NIH Guidelines* should be periodically reviewed to determine whether and how such research should be explicitly addressed.

It is the responsibility of the institution and those associated with it to adhere to the intent of the *NIH Guidelines* as well as to its specifics. Therefore, each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all research with recombinant or synthetic nucleic acid molecules conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines*. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant or synthetic nucleic acid molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

Section II–A–3. Comprehensive Risk Assessment

Currently, the risk assessment framework of the NIH Guidelines uses the Risk Group (RG) of the parent organism as a starting point for determining the necessary containment level. For example, genetic modifications of a Risk Group 3 organism (defined as agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available) would generally be carried out at Biosafety Level 3 (BL3) containment, but the containment level might be raised or lowered depending on the specific construct and the experimental manipulations. The RAC concluded that the current risk assessment framework under the *NIH Guidelines* can be effectively applied to assess the biosafety risks of experiments with synthetic nucleic acids. However, additional language was proposed to provide further guidance for evaluating synthetic biology research, which has the potential to create complex, novel organisms for which identification of a parent organism may be more difficult or may not be as relevant to the risk assessment as it is with more traditional recombinant organisms. The risk assessment may also be complicated by the limitations in predicting function from sequence(s), as recently addressed in a report by the Committee on Scientific Milestones for the Development of Gene-Sequence-Based Classification System for the Oversight of Select Agents, National Research Council, *Sequence-Based Classification of Select Agents: A Brighter Line,* ISBN–10: 0–309–15904–0. Further complications may also result from synergistic effects caused by combining sequences from different sources in a novel context.

NIH/OBA received one comment on its proposed revisions to Section II–A–3. The comment asked for clarification of the meaning of the term ''chimera'' because it is not currently used in the *NIH Guidelines*. The term was meant to capture the concept that with the advent of more sophisticated synthetic techniques, a complex organism may be created using nucleic acid sequences from multiple sources. For clarity, this wording will be used in lieu of the term ''chimera.''

Section II–A–3 Comprehensive Risk Assessment currently states:

> In deciding on the appropriate containment for an experiment, the initial risk assessment from Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard,* should be followed by a thorough consideration of the agent itself and how it is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: Virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V–B, *Footnotes and References of Sections I–IV*). A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II–B, *Containment*). The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant DNA experiments described in Sections III–A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation; III–B, Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation; III–C, Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and NIH/OBA Registration Before Initiation; III–D, Experiments that Require Institutional Biosafety Committee Approval Before Initiation.*
>
> Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level 2 (BL2) containment (see Section II–B); however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.
>
> Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult the applicable Occupational Safety and Health Administration (OSHA) regulation, 29 CFR 1910.1030, and OSHA publications, e.g., OSHA 3186–06R (2003 revised). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV-or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.
>
> Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features not addressed in *Biosafety in Microbiological and Biomedical Laboratories* (see Section V–C, *Footnotes and References of Sections I through IV*). For information regarding the importation, possession, or use of these agents see Section V–G and V–H, *Footnotes and References of Sections I through IV.*

The first paragraph is being revised to clarify that the assignment of an organism to a Risk Group in Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard,* is based on a risk assessment and identification of the Risk Group of the parent organism. The first paragraph is amended as follows:

> In deciding on the appropriate containment for an experiment, the first step is to assess the risk of the agent itself. Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard,* classifies agents into Risk Groups based on an assessment of their ability to cause disease in humans and the available treatments for such disease. Once the Risk Group of the agent is identified, this should be followed

by a thorough consideration of how the agent is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: Virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V–B, *Footnotes and References of Sections I–IV*).

The following new paragraphs will then be inserted:

While the starting point for the risk assessment is based on the identification of the Risk Group of the parent agent, as technology moves forward, it may be possible to develop an organism containing genetic sequences from multiple sources such that the parent agent may not be obvious. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest Risk Group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The Principal Investigator and Institutional Biosafety Committee must also be cognizant that the combination of certain sequences in a new biological context may result in an organism whose risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II–B, *Containment*). The appropriate containment level may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant or synthetic nucleic acid experiments described in Sections III–A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation;* III–B, *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation;* III–C, *Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and NIH/OBA Registration Before Initiation;* and III–D, *Experiments that Require Institutional Biosafety Committee Approval Before Initiation.*

Section III–A–1. Major Actions under the *NIH Guidelines*

In reviewing the *NIH Guidelines* and the different levels of review required for each category of experiment, the RAC determined that it is important also to evaluate the class of experiments that require the highest level of review: Both RAC review and NIH Director approval. In doing so, it was determined that the language for Section III–A–1–a of the *NIH Guidelines* (research involving the introduction of drug resistance into a microorganism) may not capture all of the experiments that warrant this heightened review. Moreover, given the change in the use of antibiotics and the public health problems raised by the emergence of multidrug resistant bacterial strains, clearly defining those experiments that require heightened review is a public health priority.

Section III–A–1–a currently states:

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.

In March 2009, NIH/OBA proposed to remove the phrase *not known to acquire the trait naturally* in order to allow some flexibility in review of experiments that may raise public health concern but for which there may be low levels of antibiotic resistance in the community. For example, only a small number of vancomycin-resistant *Staphylococcus* aureus strains have been isolated (B.P. Howden, *et al.* , Reduced Vancomycin Susceptibility in Staphylococcus aureus, including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection and Clinical Implications. *Clinical Microbiology Reviews.* 32(1), 99–139 (2010)). However, as there are only a limited number of antibiotics with which to treat these multidrug resistant *S. aureus* strains, the use of vancomycin resistance as a marker could raise public health concerns. Another example would be the use of ciprofloxacin resistance as a marker for *Neisseria meningitidis.* Again, there are a small number of documented cases of resistance, but ciprofloxacin remains the primary drug for post-exposure prophylaxis (H.M. Wu, *et al.,* Emergence of Ciprofloxacin-resistant *Neisseria meningitides* in North America. *N. Engl. J. Med.* 360(9), 886–92 (2009)).

In the March 2009 **Federal Register** notice, Section III–A–1–a was proposed to be amended as follows:

The deliberate transfer of a drug resistance trait to microorganisms, 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. 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.

In response to this proposed change in the language to Section III–A–1, NIH/OBA received a total of 36 written comments. Most either specifically noted their concurrence with comments from the American Society for Microbiology (ASM) or substantively concurred with ASM's comment. ASM commented that based on their interpretation of the proposed language the net effect would be to broaden substantially the scope of research that would be subject to the requirements of Section III–A–1–a and ''have a chilling impact on microbiological research where antibiotic resistance is routinely used in molecular and genetic studies.'' The ASM did agree that whether an organism is ''known to acquire the trait naturally'' is not always the critical factor in evaluating the safety of the experiment. ASM further stated that broadening the range of concern to include consideration of possible rare uses of an antibiotic that is not the ''drug of choice'' will only confound the work of the IBCs.

Other commenters noted that it was the overuse and likely misuse use of antibiotics throughout the world that pose a much greater and better documented public health threat through the development of highly resistant organisms that are capable of surviving outside of a laboratory. They noted that this threat is distinct from the laboratory setting as many laboratory-generated strains may not have a

selective advantage outside the laboratory and, even if there were inadvertent release, may not become a public health risk. Some comments suggested adding qualifiers to narrow the scope of the proposed section. For example, one commenter suggested the addition of the word ''reasonably'' to the concept of whether the transfer of drug resistance could compromise the ability to treat disease. Another commenter suggested that a list of criteria be developed that could be considered when a determination is made as to whether the transfer of a drug resistance trait could compromise public health. An additional commenter suggested that a list of ''acceptable'' transfers of drug resistance be incorporated into the *NIH Guidelines*.

Other comments revealed some potential misinterpretation of what constitutes research that falls under Section III–A–1–a. For clarification, NIH/OBA notes that transfer of a drug resistance trait to any non-pathogenic organism is not subject to the requirements of Section III–A–1–a of the *NIH Guidelines*, and transfer of resistance to a drug that is not currently used to treat disease caused by a pathogenic organism is not subject to review under Section III–A–1–a. These experiments, however, may be subject to other portions of the *NIH Guidelines*.

The changes proposed in the March 2009 **Federal Register** notice were further discussed at the public consultation on June 23, 2009. The panel of experts generally agreed that public health concerns may be raised by the use of certain antibiotic markers in pathogens that have resistance to a number of antibiotics, for example the use of vancomycin resistance as a marker in *S. aureus.* However, they concluded that these concerns could be adequately addressed by the IBC by requiring appropriate containment. The experts at the June 23, 2009, meeting agreed with ASM's observation that the safety of an experiment is not dictated solely by whether the organism can naturally acquire the resistance trait, i.e., an organism resistant to that drug has been found outside of a laboratory setting. Nonetheless, the consensus was that the original language should be maintained. They noted that there was no evidence that this section had failed to protect the public health. They also noted that once resistance has occurred in the microbial community outside of a laboratory setting, the use of such strains in a contained laboratory environment poses no additional risk to public health. Therefore, only those experiments that propose to introduce resistance to a therapeutic drug, when such resistance does not yet exist in the community, should require both RAC review and NIH Director approval. As to whether a single documented case of drug resistance is sufficient to allow this work to proceed without the necessity of RAC review and NIH Director approval, at least one expert noted that when there is a single case report, it is naïve to believe that there is only a single clinical isolate with that resistance trait. There are probably dozens or hundreds of isolates that were never reported and more that are undetected. The point is that once resistance occurs naturally, as opposed to in a laboratory setting, it is likely to occur again if acquisition of the antibiotic resistance confers a survival advantage upon the organism.

The introduction of a drug resistance trait into organisms in a laboratory setting when there are organisms outside the laboratory with this same drug resistance trait is fundamentally different than creating a novel drug resistant strain that does not exist outside of the lab. While one expert commented initially that the focus should be on resistance patterns in the U.S., others did not agree that such a limited perspective was warranted. There was consensus that there should be good documentation that this resistance exists outside of a laboratory setting and a single case report may need to be confirmed. Reports of clinical or environmental isolates should be the source of documentation of resistance.

In sum, this section of the current *NIH Guidelines* appears to protect public health adequately. There may indeed be some experiments that raise important public health considerations but would not qualify as Major Actions because there is a low level of documented resistance to the drug that will be used for selection. However, it was not possible to develop clear and easily interpretable criteria for identifying such experiments. The solution proposed was to encourage IBCs to consult with NIH/OBA and for NIH/OBA to consult with the RAC as needed when there is an experiment that does not meet the criteria for Section III–A–1–a but nonetheless raises important public health questions.

There were very few comments on the proposed language regarding analyzing subpopulations in determining the therapeutic usefulness of any antibiotic. However, there was some concern that this language might capture all antibiotics that could possibly be used rather than being limited to those antibiotics that were used clinically. Additional concern was raised about focusing on antibiotics that are not commonly used in the U.S. and therefore whether the definition of therapeutically useful should be limited to U.S. practice.

The intent of the proposed clarification regarding what is a therapeutically useful drug was not meant to expand the requirement for RAC review and NIH Director approval to all antimicrobials that might exhibit *in vitro* activity against a microorganism, but rather to focus on those that are used clinically as first or second line therapies in certain populations. The additional language was intended to raise awareness that the analysis of whether a drug is therapeutically useful needs to include consideration of certain subpopulations, in particular children and pregnant women, as many antibiotics may not be appropriate for these specific populations. With respect to antibiotics not used in the U.S., to the extent that certain pathogens have extensive impact on international populations, it is prudent to consider the antibiotic of choice in countries in which this pathogen causes disease. For example, as background to the discussion of whether the transfer of chloramphenicol resistance to *Rickettsia typhi* should be reviewed under Section III–A–1–a, the investigators noted that chloramphenicol is rarely used in the U.S. to treat disease caused by this organism. However, as this disease has considerable impact worldwide, and in particular in many developing countries in which chloramphenicol is used, this antibiotic was considered to be a therapeutically useful drug.

NIH/OBA agrees with the comments stating that the phrase ''not known to acquire the trait naturally'' serves to identify the majority of experiments that potentially pose higher risk to public health, and therefore this language will be retained. One clarification to the language was suggested by the RAC. Section III–A–1–a currently states that 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 use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC.'' As the introduction of a drug resistance trait would normally eliminate *that drug* as a therapeutic option, the analysis of whether this section applies has focused on whether the acquisition of the resistance trait by that microorganism will compromise the ability to control disease using alternative drugs. Therefore, the wording has been clarified as follows:

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 ability to control that disease agent in humans, veterinary medicine, or agriculture, will be reviewed by the RAC.

While there was consensus that this language adequately protected public health for many years and served the scientific community, there was acknowledgement that the mere fact that resistance to a drug has been documented does not necessarily mean that there are no potential public health concerns raised by use of that drug resistance trait in that microorganism. These concerns may be handled by imposing appropriate containment and other occupational health measures. In some cases, an IBC may have adequate expertise from members with training in infectious diseases to assess these risks and adopt appropriate measures, but because other IBCs may not have that same expertise, providing a mechanism for consultation with NIH/OBA or the RAC would be helpful. In order to emphasize the fact that part of NIH/OBA's role is to assist IBCs and other interested parties in evaluating containment for recombinant and synthetic nucleic acid research, the following will be added to Section III–A–1–a. This statement is a slight modification to that found currently in Section IV–C–3 (Roles and Responsibilities of the Office of Biotechnology Activities) of the *NIH Guidelines*.

At the request of an IBC, NIH/OBA will make a determination regarding whether a specific experiment 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. IBCs may also consult with NIH/OBA regarding experiments that do not meet the requirements of Section III–A–1–a but nonetheless raise important public health issues. NIH/OBA will consult, as needed, with one or more experts, which may include the RAC.

With respect to the comments about providing a list of drugs that are clinically useful for a particular disease or to generate a list of allowable transfers, inclusion of such information in the *NIH Guidelines* is not appropriate. The drugs of choice for diseases are often updated, and NIH/OBA follows the recommendation of the leading medical textbooks and medical literature. Information on where to obtain such guidance is already included in a Frequently Asked Questions document on NIH/OBA's website under IBC Information *http://oba.od.nih.gov/rdna_ibc/ibc.html.* Experiments involving the deliberate transfer of antibiotic resistance that present little or no risk to the environment, agriculture, or public health, should be addressed in informational guidances that are easily updated. Listing all acceptable transfers of antibiotic resistance is not feasible.

Section III–A–1–a will now state:

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 ability to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC.

Consideration should be given as 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 children or pregnant women.

At the request of an Institutional Biosafety Committee, NIH/OBA will make a determination regarding whether a specific experiment 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. An Institutional Biosafety Committee may also consult with NIH/OBA regarding experiments that do not meet the requirements of Section III–A–1–a but nonetheless raise important public health issues. NIH/OBA will consult, as needed, with one or more experts, which may include the RAC.

Section III–B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval

Once a Section III–A–I–a experiment is reviewed by the RAC and approved by the NIH Director, equivalent experiments may not need to follow the same approval process to determine the appropriate biosafety containment level for the work. A new section under Section III–B (Experiments that Require NIH/OBA and IBC Approval before Initiation) was proposed to allow NIH/OBA (rather than the NIH Director) to review and approve certain experiments deemed equivalent to those already approved by the NIH Director, providing there is no new information that would raise new biosafety or public health issues.

The following section is proposed to be added to the *NIH Guidelines*:

Section III–B–2. Experiments that have been Approved (under Section III–A–1–a) as Major Actions under the *NIH Guidelines*

Upon receipt and review of an application from the investigator, NIH/OBA may determine that a proposed experiment is equivalent to an experiment that has previously been approved 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 and pertinent information has not emerged since submission of the initial III–A–1 experiment that would change the biosafety and public health considerations 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.

Summary of Revised Language

The following provides the new language for the amended sections discussed above.

Title of the NIH Guidelines

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Section I. Scope of the NIH Guidelines

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 with naturally occurring nucleic acid molecules, and (iii) cells, organisms, and viruses containing such molecules.

Section I–B. Definition of Recombinant and Synthetic Nucleic AcidsIn the context of the *NIH Guidelines,* recombinant and synthetic nucleic acids are defined as:

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

(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.

Section I–C. General Applicability

Section I–C–1. The *NIH Guidelines* are applicable to:

Section I–C–1–a. All recombinant or synthetic nucleic acid research within the United States (U.S.) or its territories that is within the category of research described in either Section I–C–1–a–(1) or Section I–C–1–a–(2).

Section I–C–1–a–(1). Research that is conducted at, or sponsored by, an institution that receives any support for recombinant or synthetic nucleic acid research from NIH, including research performed directly by NIH.

An individual who receives support for research involving recombinant or synthetic nucleic acids must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines.*

Section I–C–1–a–(2). Research that involves testing in humans of materials containing recombinant or synthetic nucleic acids developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I–C–1–b. All recombinant or synthetic nucleic acid research performed abroad that is within the category of research described in either Section I–C–1–b–(1) or Section I–C–1–b–(2).

Section I–C–1–b–(1). Research supported by NIH funds.

Section I–C–1–b–(2). Research that involves testing in humans of materials containing recombinant or synthetic nucleic acids developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section II–A–3. Comprehensive Risk Assessment

In deciding on the appropriate containment for an experiment, the first step is to assess the risk of the agent itself. Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard,* classifies agents into Risk Groups based on an assessment of their ability to cause disease in humans and the available treatments for such disease. Once the Risk Group of the agent is identified, this should be followed by a thorough consideration of how the agent is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V–B, *Footnotes and References of Sections I–IV*).

While the starting point for the risk assessment is based on the identification of the Risk Group of the parent agent, as technology moves forward, it may be possible to develop an organism containing genetic sequences from multiple sources such that the parent agent may not be obvious. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest Risk Group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The Principal Investigator and Institutional Biosafety Committee must also be cognizant that the combination of certain sequences in a new biological context may result in an organism whose risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II–B, *Containment*). The appropriate containment level may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant or synthetic nucleic acid experiments described in Sections III–A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation; III–B, Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation; III–C, Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and NIH/OBA Registration Before Initiation; and III–D, Experiments that Require Institutional Biosafety Committee Approval Before Initiation.*

Section III–A–1. Major Actions under the NIH Guidelines

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 ability to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC.

Consideration should be given as 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 children or pregnant women.

At the request of an Institutional Biosafety Committee, NIH/OBA will make a determination regarding whether a specific experiment 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. An Institutional Biosafety Committee may also consult with NIH/OBA regarding experiments that do not meet the requirements of Section III–A–1–a but nonetheless raise important public health issues. NIH/OBA will consult, as needed, with one or more experts, which may include the RAC.

Section III–B–2. Experiments that have been Approved (under Section III–A–1–a) as Major Actions under the NIH Guidelines

Upon receipt and review of an application from the investigator, NIH/OBA may determine that a proposed experiment is equivalent to an experiment that has previously been approved 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 and pertinent information has not emerged since submission of the initial III–A–1 experiment that would change the biosafety and public health considerations 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.

Section III–C–1.

Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants

Human gene transfer is the deliberate transfer into human research participants of either:

Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or

Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:

a. Contain more than 100 nucleotides; or

b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or

c. Have the potential to replicate in a cell; or

d. Can be translated or transcribed.

No research participant shall be enrolled (see definition of enrollment in Section 1–E–7) until the RAC review process has been completed (see Appendix M–I–B, *RAC Review Requirements*).

Section III–F. Exempt Experiments

The following recombinant or synthetic nucleic acid molecules are exempt from the

and registration with the Institutional Biosafety Committee is not required; however, other federal and state standards of biosafety may still apply to such research (for example, the Centers for Disease Control and Prevention (CDC)/NIH publication Biosafety in Microbiological and Biomedical Laboratories).

Section III–F–1. Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (*e.g.* , oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an

LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III–C it is not exempt under this Section.

Section III–F–2. Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.

Section III–F–3. Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

Section III–F–4. Those that consist entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III–F–5. Those that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III–F–6. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV–C–1–b–(1)–(c), Major Actions). See Appendices A–I through A–VI, Exemptions under Section III–F–6–Sublists of Natural Exchangers, for a list of natural exchangers that are exempt from the *NIH Guidelines.*

Section III–F–7. Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

Section III–F–8. Those that do not present a significant risk to health or the environment (see Section IV–C–1–b–(1)–(c), Major Actions), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, Exemptions under Section III–F–8 for other classes of experiments which are exempt from the *NIH Guidelines.*

Section IV–A. Policy

The safe conduct of experiments involving recombinant or synthetic nucleic acids depends on the individual conducting such activities. The *NIH Guidelines* cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The *NIH Guidelines* are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. The *NIH Guidelines* will never be complete or final since all experiments involving recombinant or synthetic nucleic acid molecules cannot be foreseen. The utilization of new genetic manipulation techniques may enable work previously conducted using recombinant means to be accomplished faster, more efficiently, or at larger scale. These techniques have not yet yielded organisms that present safety concerns that fall outside the current risk assessment framework used for recombinant nucleic acid research. Nonetheless, an appropriate risk assessment of experiments involving these techniques must be conducted taking into account the way these approaches may alter the risk assessment. As new techniques develop, the *NIH Guidelines* should be periodically reviewed to determine whether and how such research should be explicitly addressed.

It is the responsibility of the institution and those associated with it to adhere to the intent of the *NIH Guidelines* as well as to their specifics. Therefore, each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all research with recombinant or synthetic nucleic acid molecules conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines.* The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant or synthetic nucleic acid molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

Dated: August 29, 2012.

Lawrence A. Tabak,

Deputy Director, National Institutes of Health.

[FR Doc. 2012–21849 Filed 9–4–12; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Substance Abuse and Mental Health Services Administration

Current List of Laboratories and Instrumented Initial Testing Facilities Which Meet Minimum Standards To Engage in Urine Drug Testing for Federal Agencies

AGENCY: Substance Abuse and Mental Health Services Administration, HHS.

ACTION: Notice.

SUMMARY: The Department of Health and Human Services (HHS) notifies Federal agencies of the Laboratories and Instrumented Initial Testing Facilities (IITF) currently certified to meet the standards of the Mandatory Guidelines for Federal Workplace Drug Testing Programs (Mandatory Guidelines). The Mandatory Guidelines were first published in the **Federal Register** on April 11, 1988 (53 FR 11970), and subsequently revised in the **Federal Register** on June 9, 1994 (59 FR 29908); September 30, 1997 (62 FR 51118); April 13, 2004 (69 FR 19644); November 25, 2008 (73 FR 71858); December 10, 2008 (73 FR 75122); and on April 30, 2010 (75 FR 22809).

A notice listing all currently certified Laboratories and Instrumented Initial Testing Facilities (IITF) is published in the **Federal Register** during the first week of each month. If any Laboratory/IITF's certification is suspended or revoked, the Laboratory/IITF will be omitted from subsequent lists until such time as it is restored to full certification under the Mandatory Guidelines.

If any Laboratory/IITF has withdrawn from the HHS National Laboratory Certification Program (NLCP) during the past month, it will be listed at the end and will be omitted from the monthly listing thereafter.

This notice is also available on the Internet at *http://www.workplace.samhsa.gov* and *http://www.drugfreeworkplace.gov*.

FOR FURTHER INFORMATION CONTACT: Mrs. Giselle Hersh, Division of Workplace Programs, SAMHSA/CSAP, Room 2–1042, One Choke Cherry Road, Rockville, Maryland 20857; 240–276–2600 (voice), 240–276–2610 (fax).

SUPPLEMENTARY INFORMATION: The Mandatory Guidelines were initially developed in accordance with Executive Order 12564 and section 503 of Public Law 100–71. The ''Mandatory Guidelines for Federal Workplace Drug Testing Programs'', as amended in the revisions listed above, requires strict standards that Laboratories and Instrumented Initial Testing Facilities (IITF) must meet in order to conduct drug and specimen validity tests on urine specimens for Federal agencies.

To become certified, an applicant Laboratory/IITF must undergo three rounds of performance testing plus an on-site inspection. To maintain that certification, a Laboratory/IITF must participate in a quarterly performance testing program plus undergo periodic, on-site inspections.

Laboratories and Instrumented Initial Testing Facilities (IITF) in the applicant stage of certification are not to be considered as meeting the minimum requirements described in the HHS Mandatory Guidelines. A Laboratory/IITF must have its letter of certification from HHS/SAMHSA (formerly: HHS/NIDA) which attests that it has met minimum standards.

In accordance with the Mandatory Guidelines dated November 25, 2008 (73 FR 71858), the following Laboratories and Instrumented Initial Testing Facilities (IITF) meet the minimum standards to conduct drug and specimen validity tests on urine specimens: