Alnylam Pharmaceuticals

Corrigan-Curay, Jacqueline (NIH/OD) [E]

From: Sara Nochur [snochur@alnylam.com]
Sent: Wednesday, April 29, 2009 8:49 PM
To: Corrigan-Curay, Jacqueline (NIH/OD) [E]
Subject: RE: Response to NIH Federal Register on Recombinant DNA

Dear Jacqueline,

I authorize the NIH Office of Biotechnology Activities to use the comments submitted by Alnylam Pharmaceuticals on April 29, 2009, that are in response to the Federal Register Notice, published March 4, 2009 (74 FR 9411) to carry out its mission, including release of all such information to the public. This consent to public disclosure specifically includes information that was labeled "confidential."

Hope this addresses the issue.

Thank you.

Sincerely,
Sara

Saraswathy (Sara) V. Nochur, Ph.D.
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Alnylam® PHARMACEUTICALS

29 April 2009

Jacqueline Corrigan-Curay, J.D., M.D.
Executive Secretary
Recombinant DNA Advisory Committee
Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, Maryland 20892–7985

Re: Comment to Federal Register Notice Vol. 74, No. 41 dated 4 March 2009 regarding Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules

Dear Dr. Corrigan-Curay,

We appreciate the opportunity to comment on the 4 March 2009 Federal Register Notice (Vol. 74, No. 41) regarding "Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules".

We recommend that since synthetic nucleic acids such as small interfering ribonucleic acids (siRNAs), antisense oligonucleotides, and microRNAs are a class of molecules that do not encode proteins or function as transcription templates for coding or non-coding RNA in mammalian cells, do not replicate in the nucleus or cytoplasm of mammalian cells, and do not integrate into mammalian host genomic DNA, they pose little biosafety risk, and therefore should be exempted from RAC and IBC review for both pre-clinical and human research. Our detailed comments are attached.

Please do not hesitate to contact me at 617-551-8393 with any questions or comments.

Thank you.

Sincerely,

Saraswathy (Sara) V. Nochur, Ph.D.
Vice President, Regulatory Affairs

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COMMENTS FROM ALNYLAM PHARMACEUTICALS ON THE PROPOSED ACTIONS UNDER THE NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

We are submitting this comment in response to the Federal Register Notice Vol. 74, No. 41 dated 4 March 2009 regarding the National Institutes of Health's Office of Biotechnology Activities: Recombinant DNA Research: Proposed Action Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), and the proposal to revise the guidelines to expand the scope to 'nucleic acid molecules made solely by synthetic means".

Introduction

Alnylam Pharmaceuticals, the leader in the development of RNA interference (RNAi) therapeutics using synthetic small interfering RNAs (siRNAs), understands the need for the NIH initiative to update the definition of recombinant DNA to reflect scientific progress made since these guidelines were first established. 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 conducted in the research laboratory and when being considered for use in human subjects, and thus should be subject to OBA registration and RAC review. In contrast, and as acknowledged in the Federal Register Notice in Section III-F-1 (4), we believe that it would be inappropriate to include certain classes of synthetic nucleic acids, including siRNAs, under the rubric of recombinant nucleic acids since they lack all of the essential properties listed above. Indeed, from a pharmacologic perspective, siRNA-based therapeutics are more closely related to traditional small molecule drugs than to recombinant nucleic acids. Therefore, we propose that they be excluded from the definition of recombinant molecules under consideration by the NIH.

The siRNA Mechanism of Action

Since the discovery of RNAi[1], the mechanism of action and associated molecular biology have been delineated in great detail[2, 3]. With siRNA therapeutics, a short piece of synthetic, linear, double stranded RNA (dsRNA; 20-30 base pairs in length) is the therapeutic agent. This dsRNA is designed such that one of the strands of the duplex is complementary to a segment of messenger RNA (mRNA) that encodes a disease-related protein. The dsRNA is bound by an enzyme complex known as the RNA Induced

Silencing Complex (RISC). RISC selects one strand of the dsRNA, and then the enzyme complex efficiently seeks out and binds to mRNAs containing a sequence that is complementary to the RNA strand loaded in RISC. When a complementary mRNA strand is found, RISC cleaves the target mRNA at a defined location, and is then free to bind and cleave another mRNA. Cleavage leads to mRNA degradation which ultimately reduces the expression of the (disease-related) protein encoded by the target mRNA.

siRNA Duplexes are Neither Expressed nor Amplified by Cells

Synthetic siRNAs cannot function as templates for protein expression. They contain neither 5' cap structures nor any other functional elements found in mRNAs. Also, they do not include any promoter or enhancer elements and, therefore, cannot be transcribed in cells. In this way, siRNAs are fundamentally distinct from most synthetic or recombinant DNA molecules which are generally designed to express proteins. In addition, these small double-stranded RNAs contain none of the cis-regulatory elements required to create additional copies of the siRNA. In short, siRNAs are not functional pieces of genetic material with expression and replicative properties.

siRNAs Cannot Integrate Into the Host Genome

One of the concerns when administering an oligonucleotide-based therapeutic is the potential for integration of the oligonucleotide fragment into the genome. Can genomic DNA be an unintended target for siRNA as a result of integration of the siRNA into the genome? This is highly unlikely, if not impossible, because there is no precedent for direct integration of an RNA molecule into genomic DNA. While it is well known that retroviruses, a class of RNA-genome viruses, can become stably integrated into the host genome, this requires the creation of a DNA copy of viral genomic RNA[4, 5]. This highly complex process is dependent upon virally-expressed factors and specific sequence elements within the viral genome, which are lacking in siRNAs. Thus, integration into the genome cannot be an unintended consequence of administering siRNA.

Impact of Formulations that Facilitate Targeting of siRNAs to Specific Tissues

The properties of the siRNA described above are not altered by formulations that allow targeting of siRNAs into particular cells or tissues. Thus, for example, when siRNAs are formulated with cationic lipids or conjugated with specific ligands, such formulations may facilitate delivery of siRNAs to specific tissues and may also allow for access to specific cells within the tissue; however, the siRNA still would operate via the RISC-

mediated RNAi mechanism and would not be able to either express or replicate in the cell, or incorporate into the genome.

The Pharmacology of siRNAs Resembles Typical Drugs

In the siRNA mechanism, all of the classic principles of pharmacology apply. Like all pharmacologic agents, the concentration of the siRNA delivered to the cell determines the magnitude of the response, and the pharmacologic response is reversed as the siRNA is cleared as a result of metabolism. The siRNAs that are in development at Alnylam are cleared like typical drugs with pharmacologic effects that reverse when the drug is cleared by excretion or metabolism. Moreover, reversal of siRNA activity has been repeatedly demonstrated in animal models including studies in non-human primates[6].

In contrast, synthetic or recombinant nucleic acid gene-therapy vectors, whose mechanism of action includes protein expression, replication and/or genomic integration, are designed to have sustained activity that is maintained indefinitely.

Conclusions

We conclude that siRNAs are more properly regarded as drugs that act on specific messenger RNAs and thereby bring about targeted protein suppression, resulting in therapeutic benefit. They do not have the issues of expression, replication and/or genomic integration that have been the reason behind the regulation of recombinant DNA products. Although siRNAs are synthetically manufactured nucleic acids, the absence of these key properties should make them exempt from the modified definition being proposed.

Thus, as a class, since synthetic nucleic acids (such as siRNA, antisense oligonucleotides, and miRNA) are molecules that do not:

- Encode proteins or function as transcription templates for coding or non-coding RNA in mammalian cells,
- Replicate in the nucleus or cytoplasm of mammalian cells, and
- Integrate into mammalian host genomic DNA,

they should be exempt from this guideline and not require RAC oversight for proposed nonclinical or clinical studies.

Further, as stated above, formulations that facilitate targeted delivery of siRNAs to tissues or cells do not alter their fundamental properties and therefore, formulated siRNAs should also be exempt from the proposed new definition.

Bibliography

1. Fire, A. et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* **391**, 806-11 (1998).
2. Dykxhoorn, D.M., Novina, C.D. & Sharp, P.A. Killing the messenger: short RNAs that silence gene expression. *Nat Rev Mol Cell Biol* **4**, 457-67 (2003).
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4. Temin, H.M. & Baltimore, D. RNA-directed DNA synthesis and RNA tumor viruses. *Adv Virus Res* **17**, 129-86 (1972).
5. Baltimore, D. Retroviruses and retrotransposons: the role of reverse transcription in shaping the eukaryotic genome. *Cell* **40**, 481-2 (1985).
6. Zimmermann, T.S. et al. RNAi-mediated gene silencing in non-human primates. *Nature* **441**, 111-4 (2006).

American Biological Safety Association (ABSA)

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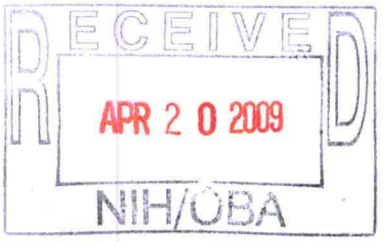
April 15, 2009



Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750 MSC 7985
Bethesda, MD 20892-7985

Dear Sir or Madam:

The American Biological Safety Association (ABSA) is an international group of biological safety professionals that is one of the world's foremost resources on biological safety practices. We have been reviewing the proposed revisions to the *"NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)"* which were announced in the Federal Register on March 4, 2009.

It is evident that these proposed revisions were given great thought and deliberation by OBA before they were released. We have begun the process of preparing our comments to them. It has become clear during our review process that the proposed revisions could have a significant impact on this type of research and could have unforeseen consequences if they are not carefully vetted.

We request that the announced comment period be extended from 60 days to at least 90 days so that we may have a greater opportunity to provide the thorough review which the proposal merits.

If you can accommodate this request, then please let us know. Thank you.

Sincerely,

Robert P. Ellis

Robert Ellis, PhD. CBSP
President
American Biological Safety Association

American Biological Safety Association (ABSA)

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RECEIVED
MAY 29 2009
NIH/OBA

Dr. Jaqueline Corrigan-Curray

Office of Biotechnology Activities

National Institutes of Health

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May 28, 2009

Dear Dr. Corrigan-Curray,

The American Biological Safety Association (ABSA) is an international group of biological safety professionals which is known as one of the world's foremost resources on biological safety practices. We have reviewed the proposed revisions to the "NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)" which were announced in the Federal Register on March 4, 2009. Please consider the comments that follow regarding this proposal.

General Comments

The addition of Institutional Biosafety Committee (IBC) reviews of experiments involving synthetic nucleic acids will require additional expertise on the local IBCs. The NIH Guidelines should reflect the need for additional expertise on the local IBCs if these reviews are to have the intended scope and benefit. The Recombinant DNA Advisory Committee (RAC) will likely face similar challenges in its national reviews.

Specific Comments

Proposed Changes to Section I-A, Section I-B: The NIH Guidelines must be reviewed carefully and thoroughly for meaning when replacing the term "recombinant DNA molecules" with "recombinant and synthetic nucleic acid molecules." Section I-A states, "In accordance with this change in the scope of the NIH Guidelines, the term, 'recombinant DNA molecules' will be replaced with 'recombinant and synthetic nucleic acid molecules.'" To be clear, the replacement text should be 'recombinant *and/or* synthetic nucleic acid molecules." Consistent use of this distinction throughout the Guidelines should help to avoid possible confusion amongst the entities seeking to implement provisions of the NIH Guidelines. An alternative would be to use the

single term, "nucleic acid" or "nucleic acids" instead of "recombinant and synthetic nucleic acid molecules" with a definition for "nucleic acid" or "nucleic acids" that makes the needed clarification.

Proposed Changes to Section I-B: Under "Definition", the proposed text states: In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as: (i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell, (ii) synthetic nucleic acid molecules that are chemically, or by other means, synthesized or amplified nucleic acid molecules that may wholly or partially contain functional equivalents of nucleotides, or (iii) molecules that result from the replication of those described in (i) or (ii) above." The phrases used in this section do not provide the clarity required to make the clear distinctions needed to effectively apply the Guidelines to research. We suggest the following alternative text:

Section I-B-a. Definition. "In the context of the NIH Guidelines DNA, RNA and synthetic nucleic acids are considered to be nucleic acids regardless of their origin.", or

Section I-B-a. Definition. "In the context of the NIH Guidelines recombinant nucleic acids (NA) are defined as molecules constructed by joining nucleic acid segments, regardless of their origin, into biochemically unique constructed molecules that can (i) replicate in a living cell or (ii) generate molecules that can replicate in a living cell."

Proposed Changes to Section 1-B: The terms "low risk" and "high risk" need further characterization as applied to synthetic, nucleic acids. Possible means by which this characterization could be accomplished would be for NIH/OBA to develop and share "Fact Sheets" regarding low-risk and high-risk experiments. These "Fact Sheets" could better define these types of experiments and provide illustrative examples of each type of experiment. Inclusion of a decision tree detailing the process that should be considered when making these distinctions would contribute additional utility of these documents.

There is another need for such "Fact Sheets." The announced scope of the revised NIH Guidelines would be inclusive of work of principal investigators (PIs) such as chemistry or engineering researchers. These PIs likely have no previous experience in conducting research that is considerate of the NIH Guidelines, and many of them may have no experience in risk assessments that are inherent to research considerate of these Guidelines. The language in any "Fact Sheets" should be in "lay language" to facilitate the understanding of these PIs regarding the NIH Guidelines. These fact sheets could also be helpful for IBC members who represent the entity's community.

Proposed Changes to Section II-A-3: The following new paragraphs are proposed by NIH to be added to the Guidelines:

"[New Paragraph] While the initial 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 a chimera in which the parent agent may not be obvious. In such cases, the risk assessment should involve 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 an analysis of the functional attributes of these sequences (e.g., sequence associated with virulence factors, pathogenicity, transmissibility, etc.). It may be prudent to first consider the highest risk group classification of any agent sequence included in the chimera. Other factors to be considered include the percentage of the genome

contributed by each of multiple parent agents, and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as predicted in the original host context.

"[New Paragraph] The IBC must also be cognizant that the combination of certain sequences 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. A new biosafety risk may occur with a chimera 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.

These paragraphs reference the terms, "chimera" and "parent agent". These terms are used for the first time in the Guidelines in these paragraphs. These terms do not have universal meaning between investigators, and the definition inconsistencies could result in differences in the application by investigators of these new provisions of the Guidelines. "Parent strain" is used frequently in the Guidelines, and it is widely and consistently recognized as being the wild-type origin from which a genetically modified organism is generated. It is proposed that the term "parent strain" be replaced with "parent agent." Chimera could be defined as: "resulting nucleic acid derived from two or more genotypically diverse parent agent nucleic acid segments."

In addition, the text which follows is suggested for consolidating these two paragraphs in a manner that focuses their meaning and intent:

"[New Paragraph] Genetically modified organisms containing two or more nucleic acid segments, regardless of origin, may necessitate a complex risk analysis. Preliminary analysis should consider the risk associated with original source sequences taking into account their virulence, pathogenicity, transmissibility, *etc*. Additional risk assessment analysis must include the additive effects of the greater of the associated risks. Because there may be unanticipated consequences of multiple genetic modifications, the possibility of greater or lower risk-hazard than expected must be considered. It may be necessary to test the final modified organisms with *in vitro and/*or *in vivo* studies under the initial assumption that they are a high-risk hazard." As a result of these risk analysis determinations, defined acceptable risks must be developed and promulgated.

<u>Comment regarding Section III-A-1</u>.
The American biological Safety Acssociation endorses the ASM comments regarding Section III-A-1.

<u>Proposed Changes to Section III-C</u>: The proposed text for this section is, "For an experiment involving the deliberate transfer of recombinant or synthetic nucleic acids into 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)."

Use of the term, "human gene transfer" is appropriate in the existing NIH Guidelines. However, its use would not cover "non-coding" sections such as shRNA and antisense RNA as well as other current and future structures. All of these constructs cannot be considered to be genes. Use of the terms "therapeutic nucleic acid transfer" or "clinical recombinant nucleic acid" would be more appropriate, or consider the use of another term that is inclusive of synthetic nucleic acids in its scope. Since the term, "human gene transfer" is used multiple times in the Guidelines, any alternative term should be consistently used throughout the Guidelines and not just in this

section. This text should also note coverage of the Guidelines in veterinary applications of these materials.

The proposed changes to the NIH Guidelines demonstrate a clear case for the need for applied biological safety research and training across a broad range of recombinant and synthetic nucleic acid issues. Research results would provide evidence-based data and guidance that will help in determining risk assessments and appropriate training to different user groups. NIH or OBA should seek funding to conduct research to characterize risks associated with investigations considerate of the NIH Guidelines, as well as funding to help train investigators and staff at all levels to more safely and effectively conduct these investigations.

ABSA would welcome the opportunity to assist OBA in any restructuring and revisions to the NIH Guidelines that may be under consideration. Many technical and administrative issues have changed since the NIH Guidelines were initially drafted. Restructuring and revisions of this document could facilitate its use and could better address some of the current issues which researchers are now facing.

We appreciate this comment opportunity for these proposed revisions.

Sincerely,

Robert P. Ellis

Robert Ellis, PhD. CBSP

President
American Biological
Safety Association

American Society of Gene Therapy (ASGCT)

AMERICAN SOCIETY of GENE THERAPY

Office of Biotechnology Activities
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Re: Comment on Federal Register Notice of March 4, 2009:
Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

The American Society of Gene and Cell Therapy is writing in response to the proposed changes in the NIH guidelines for research involving recombinant DNA molecules. Our specific comments and concerns are related to the proposed changes to include oversight of clinical trials involving oligonucleotides, and small RNA based therapeutics. Specifically, we would like to comment on section 3F-1.

(4) For human gene transfer research, are there classes of non-replicating molecules that should be exempt due to lower potential risks (e.g., antisense RNA, RNAi, etc.)? If so, what criteria should be applied to determine such classes?

The Society does not believe these categories of nucleic acid based therapeutics requires RAC review based on the nature of the agents and the historical use of drugs targeting DNA.

The therapeutic use of recombinant DNA seeks to permanently or transiently alter the genetic make-up of cells within an individual. In contrast, nucleic acid based therapeutics do not alter the genetic make-up of an individual, do not express native or foreign proteins, and as such do not pose the same ethical and safety risks associated with traditional gene therapy approaches.

Importantly, these compounds present a public and patient risk that is at or below the risk associated with commonly used drugs, including chemotherapeutic agents which alter DNA in a non-selective manner.

We base our opinion on the following three sub-points :

1) Antisense oligonucleotides and small RNAs do not contain an amplification cascade. Unlike vectors that can express an RNA and/or protein, there is no intent to provide a molecule which will amplify itself and/or an additional effector molecule.
2) The intent of oligonucleotide DNAs and small RNAs is not to alter chromosomal DNA.
3) Antisense oligonucleotides and small RNAs are formulated in a manner similar to approved drugs and small molecules and are amenable to well established

pharmacologic and toxicological testing. As such, they do not pose the same safety risks associated with traditional gene therapy approaches that can be difficult to test in traditional pharm/tox assays.

Two additional points to be addressed are the potential for DNA damage and specialized formulation of these drugs. While the majority of oligonucleotides do not target DNA, certain compounds are intended to alter the DNA sequence (such as Triplex forming oligonucleotides and certain chromosomal targeted DNAs). Disrupting DNA is the mode of action of a large number of commonly used, FDA-approved, drugs (including 5 -flurouracil, Daunorubicin, BCNU, hydroxyurea, acyclovir, and ganciclovir). Several of these drugs are designed to cause chromosomal damage that will lead to cell apoptosis. None of these drugs require RAC approval for clinical use despite their intent to cause DNA changes. Moreover, the advantage of oligonucleotides that disrupt DNA is that it provides specific rather than global DNA damage and should increase the safety profile of therapy compared to the current FDA-approved drugs.

Formulation has also been discussed, specifically the use of lipids to deliver oligonucleotides and small RNAs. FDA has approved lipids for drug delivery, including the commonly use chemotherapeutic agent doxorubicin and the antifungal agent Amphotericin B. Lipid mediated drug delivery is well established and does not require special NIH oversight.

In summary, the ASGCT believes the nucleic acid based therapies discussed above should be exempt from RAC review. These would include single-stranded antisense oligonucleotides (DNA, RNA, or novel nucleic acid chemistry), or short double-stranded RNAs. These molecules do require rigorous testing in the manner established and regulated by the US Food and Drug Administration, an organization that remains in the best position to oversee the development of this new class of drugs.

Respectfully yours,

Mary Dean

Mary Dean
Executive Director, ASGCT

cc: ASGCT Board of Directors

American Society for Microbiology (ASM)

AMERICAN
SOCIETY FOR
MICROBIOLOGY

Public and Scientific Affairs Board

April 9, 2009

Office of Biotechnology Activities
National Institutes of Health
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Subject: Response to request for comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register,* 9411-21

The American Society for Microbiology (ASM) is submitting the following comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register*, 9411-21:

Revision to Section III-A-1 Major Actions Under the NIH Guidelines. The proposed revised section states that all experiments involving "the deliberate transfer of a drug resistance trait to a microorganism, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture," will receive RAC review and NIH Director approval. The current NIH Guidelines state that if the microorganism is known to acquire the trait naturally, then transfer of the drug resistance may not need RAC review. The NIH is now proposing to delete the phrase "that are not known to acquire the trait naturally," in Section III-A-1.

Further discussion of this stringent review policy and assessment of the presumed risk to the public and the environment posed by antibiotic resistance markers in basic and pathogenic bacteriology research is needed. If interpreted literally, as it likely will be, this language could have a chilling impact on microbiological research where antibiotic resistance is routinely used in molecular and genetic studies. Given that no documented harm has come from laboratory research using antibiotic resistance markers, the question must be asked as to why a change in the Guidelines is warranted. Any change in Section III-A-1 should clarify and narrowly focus on areas of concern. The proposed language does the opposite. By broadening the activities that require approval, it will have an adverse impact on microbiological research and public health.

The stated purpose of the revision to Section III–A–1 is to clarify the current guidelines for local Institutional Biosafety Committees (IBCs). The NIH Guidelines are based on the premise that local oversight is the best approach to biosafety. However, the work of the IBCs will be more complicated if the proposed change is adopted. While we agree that whether or not an organism acquires the trait naturally is not the critical factor in evaluating the safety of the experiment,

broadening the range of concern to include consideration of possible rare uses of an antibiotic that is not a ‘‘drug of choice,’’ will only confound the work of the IBCs.

Selectable antibiotic resistance markers introduced into bacteria via plasmids, transposons, or by homologous recombination are the most conventional, versatile, and widely used tools in the study of bacterial pathogenesis and bacterial physiology. Such antibiotic resistance genes have been used to replace or inactivate bacterial genes to elucidate key physiological or pathogenic traits. Such mutant traits are then complemented with a replacement copy of the gene borne on a plasmid that is maintained under antibiotic selection, an essential step in fulfillment of Molecular Koch's Postulates. The applications for antibiotic resistance selection are so numerous that it is impossible to envision the study of bacterial genetics without the use of antibiotic selection markers. The real question is whether such antibiotic resistance markers pose an actual risk in treatment of infections with the bacterial strains and pathogens we study.

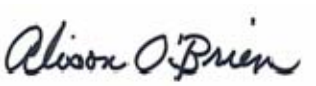
Previously, researchers took into consideration the likelihood that the selection agent would be clinically used as a therapy for infection. However, concerns about multiply resistant agents of all types, the "drugs of choice" and the threat to the public posed by resistant bacteria are more difficult to determine. Nonetheless, the emergence of multiple drug resistance in bacteria is universally regarded as a product of the indiscriminant use of antibiotics in humans and in agriculture worldwide. Therefore, bacteriologic strains developed during the course of basic scientific research and tested in vitro or in vivo in laboratory animal models are relatively unlikely to pose any threat to the population or environment at large.

Given the value of antibiotics in the study of bacteria, and the mechanisms already in place through other federal regulations to protect the public from agents in the research laboratory environment, we consider this revision to the RAC Guidelines as ill-defined in purpose and counterproductive to the generation of helpful science in the interest of public health.

The ASM supports the proposed revisions to Section I-B, Basic Research with Recombinant and Synthetic Nucleic Acids, which clarifies the applicability of the NIH Guidelines to research with synthetic nucleic acids, and Section III-E-1, Experiments Involving DNA Molecules Containing No More Than One Half of the Genome of Any Eukaryotic Virus, which changes the level of review for recombinant or synthetic experiments involving more than half but less than two thirds of the genome of certain viruses.

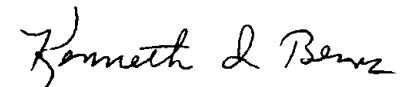
We appreciate the opportunity to comment on the proposed changes to the RDNA Guidelines.

Sincerely,



Alison O'Brien, Ph.D.
President, ASM

Ronald M. Atlas, Ph.D.
Co-Chair, Committee on Biodefense



Kenneth I. Berns, M.D., Ph.D.
Co-Chair, Committee on Biodefense

Association of American Medical Colleges (AAMC)

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May 3, 2009

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Subject: Response to Proposed Revisions in the NIH Guidelines for Research Involving Recombinant DNA Molecules, 74 FR 9411

Dear Sir/Madam:

The Association of American Medical Colleges (AAMC) is pleased to submit the following comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register.

AAMC is a not-for-profit association representing all 130 accredited U.S. medical schools; nearly 400 major teaching hospitals and health systems, including 68 Department of Veterans Affairs medical centers; and nearly 90 academic and scientific societies. The AAMC member medical schools and teaching hospitals collectively perform about 60 percent of all extramural research sponsored by the NIH, and a significant portion of the life sciences research supported by other agencies.

We share the concerns of the American Society for Microbiology (ASM) on the proposed revision to Section III-A-1 Major Actions Under the NIH Guidelines. The proposed revised section states that all experiments involving "the deliberate transfer of a drug resistance trait to a microorganism, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture," must receive RAC review and require NIH Director approval. The current NIH Guidelines state that if the microorganism is known to acquire the trait naturally, then transfer of the drug resistance may not need RAC review. The NIH is now proposing to delete the phrase "that are not known to acquire the trait naturally" in Section III-A-1.

We have heard from a number of institutions and faculty members concerned that the proposed revision will have a detrimental impact on research protocols where introduction of antibiotic resistance markers into bacteria is currently permitted and routinely used. This appears to be the case in a very large number of molecular and genetic studies. There is no evidence that any NIH

harm has resulted from the use of antibiotic resistance markers in compliance with current NIH Guidelines and there is no evidence that such use poses an actual risk in the future.

We strongly urge that this proposed revision be withdrawn and that the Recombinant DNA Advisory Committee hold a new public in-depth review of the scientific, safety, and ethical dimensions of this proposed change. This will allow the Committee to better understand the implications of the change and why the affected research community views this proposal with such alarm.

We have no objection to the other changes to the guidelines proposed in the March 4 notice.

We appreciate the opportunity to comment on this matter. Any questions should be directed to Tony Mazzaschi, 202-828-0059 or tmazzaschi@aamc.org.

Sincerely,

Darrell G. Kirch

Darrell G. Kirch, M.D.

Daniel R. Kuritzkes, M.D.
Director of AIDS Research
Professor of Medicine

Brigham and Women's Hospital

The opinions expressed in this memo are those of the author and are not necessarily the official position of Brigham and Women's Hospital.

BRIGHAM AND WOMEN'S HOSPITAL
HARVARD MEDICAL SCHOOL

Daniel R. Kuritzkes, M.D.
Director of AIDS Research
Professor of Medicine

April 26, 2009

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892-7985

Re: Response to request for comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register, 9411-21

I am submitting the following comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register, 9411-21:

I strongly support the comments submitted by the American Society for Microbiology regarding the proposed changes. The proposal to delete the phrase "that are not known to acquire the trait naturally," from Section III-A-1 of the current NIH Guidelines is ill-conceived and poorly justified. The proposed wording will have no positive effect on public safety, but will have a chilling effect on nearly every aspect of molecular biology research, which relies heavily on the use of bacterial resistance markers in the performance of even the simplest recombinant DNA experiments. Moreover, this regulation would paralyze the functioning of most institutional biosafety committees the NIH Recombinant DNA Advisory Committee, who would be inundated by tens of thousands of protocols for review.

Selectable antibiotic resistance markers introduced into bacteria via plasmids, transposons, or by homologous recombination are the most conventional, versatile, and widely used tools in the study of bacterial pathogenesis and bacterial physiology. Such antibiotic resistance genes have been used to replace or inactivate bacterial genes to elucidate key physiological or pathogenic traits. Such mutant traits are then complemented with a replacement copy of the gene borne on a plasmid that is maintained under antibiotic selection, an essential step in fulfillment of Molecular Koch's Postulates. The applications for antibiotic resistance selection are so numerous that it is impossible to envision the study of bacterial genetics without the use of antibiotic selection markers. The real question is whether such antibiotic resistance markers pose an actual risk in treatment of infections with the bacterial strains and pathogens we study.

In addition to these concerns regarding bacterial genetics, the same burden is imposed by excessive regulatory zeal and false safety concerns regarding the introduction of drug resistance mutations into viruses. My own work focuses on drug resistance to antiretrovirals. The only

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purpose of this work is to study resistance to clinically relevant drugs. Every single experiment performed in my laboratory would require review by the NIH Director under the proposed rule change. Yet, there is zero risk to the general public that viruses we create in the lab would pose a risk to treatment since 1) we take substantial measures to prevent anyone in the lab from becoming HIV infected in the workplace, and 2) should an accidental transmission occur, the opportunities for dissemination to the general public would be severely limited given the requirement for intimate interpersonal contact for HIV transmission.

Engineering drug resistance into influenza might be cause for greater concern, but the appropriate solution is to require as a matter of routine the use of appropriate containment and biosafety practices for such experiments *as a class*, rather than requiring individual review.

I appreciate the opportunity to comment on the proposed changes to the RDNA Guidelines.

Sincerely,

Daniel R. Kuritzkes, MD

Barbara J.B. Johnson, Ph.D
Bacterial Diseases Branch
Division of Vector-Borne Infectious Diseases

Centers for Disease Control and Prevention

From: Johnson, Barbara J. (CDC)
Sent: Wednesday, April 29, 2009 6:58 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: Beard, Charles B. (CDC); Bearden, Scott (CDC); Gilmore, Robert D. (CDC)
Subject: Proposed Action on RDNA Experiments Involving Drug Resistant Traits

To Whom It May Concern:

I share the view of the ASM that the proposed action on RDNA experiments involving drug resistant traits would unnecessarily jeopardize basic research. This paragraph from the ASM response is particularly apt: Selectable antibiotic resistance markers introduced into bacteria via plasmids, transposons, or by homologous recombination are the most conventional, versatile, and widely used tools in the study of bacterial pathogenesis and bacterial physiology. Such antibiotic resistance genes have been used to replace or inactivate bacterial genes to elucidate key physiological or pathogenic traits. Such mutant traits are then complemented with a replacement copy of the gene borne on a plasmid that is maintained under antibiotic selection, an essential step in fulfillment of Molecular Koch's Postulates. The applications for antibiotic resistance selection are so numerous that it is impossible to envision the study of bacterial genetics without the use of antibiotic selection markers. The real question is whether such antibiotic resistance markers pose an actual risk in treatment of infections with the bacterial strains and pathogens we study.

There is ample evidence that antibiotic resistance is evolving in organisms in the environment due to overuse of antibiotics agriculture, veterinary, and human medicine. If further regulations are required to protect public and animal health, these are the sectors to focus on. Prohibiting or delaying work using selectable markers that are not front-line drugs in the treatment of the pathogens that we study is not in the interest of science. I am not aware of any evidence that the proposed guideline modifications are necessary for human safety or protection of the biosphere.

Respectfully submitted,

Barbara J.B. Johnson, Ph.D.
Bacterial Diseases Branch
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The opinions expressed in this memo are those of the author and are not necessarily the official position of the Centers for Disease Control and Prevention.

Kathleen F. Keyes, MS, SM(NRCM), BBSP
CCID Safety Manager
CCID Laboratory Quality and Safety Management

Centers for Disease Control and Prevention

The CDC IBC Institutional Biosafety Committee (IBC), User's Group, and CDC Internal Select Agents Program are submitting the following comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register, 9411-21:

Of particular concern to our IBC members and User's Group is the proposed revision to Section III-A-1 (Major Actions Under the NIH Guidelines) that states that all experiments involving "the deliberate transfer of a drug resistance trait to a microorganism, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture," will receive RAC review and NIH Director approval. The current NIH Guidelines state if the microorganism is known to acquire the trait naturally, then transfer of the drug resistance may not need RAC review. The NIH is now proposing to delete the phrase "that are not known to acquire the trait naturally," from section III-A-1.

Our IBC members and PIs who submit protocols for review to us share the view articulated by ASM that the proposed action on rDNA experiments involving drug resistant traits would unnecessarily jeopardize basic research. They feel the following paragraph from the ASM response is particularly apt: "Selectable antibiotic resistance markers introduced into bacteria via plasmids, transposons, or by homologous recombination are the most conventional, versatile, and widely used tools in the study of bacterial pathogenesis and bacterial physiology. Such antibiotic resistance genes have been used to replace or inactivate bacterial genes to elucidate key physiological or pathogenic traits. Such mutant traits are then complemented with a replacement copy of the gene borne on a plasmid that is maintained under antibiotic selection, an essential step in fulfillment of Molecular Koch's Postulates. The applications for antibiotic resistance selection are so numerous that it is impossible to envision the study of bacterial genetics without the use of antibiotic selection markers. The real question is whether such antibiotic resistance markers pose an actual risk in treatment of infections with the bacterial strains and pathogens we study". Our IBC members and PIs believe there is ample evidence that antibiotic resistance is evolving in organisms in the environment due to overuse of antibiotics agriculture, veterinary, and human medicine, not from biomedical research. If further regulations are required to protect public and animal health, these are the sectors to focus on. Prohibiting or delaying work using selectable markers that are not front-line drugs in the treatment of the pathogens that we study is not in the interest of science. We are not aware of any evidence that the proposed guideline modifications are necessary for human safety or protection of the biosphere.

This proposed change to the *NIH Guidelines* will most certainly have an impact on how we at the CDC review proposals in our IBC (i.e., changes in section III-A-1-a that will require RAC review for any transfer of an antibiotic resistance gene if that antibiotic is currently used in disease treatment) and will certainly slow down protocol approval and new research. Note, too, that these would be considered "restricted experiments" by definition in the select agent regulations, if select agents are involved, and as such would also require independent approval by the HHS or USDA Secretary (whichever rule is applicable). We have defined this in our CDC Internal Select Agent Program User's Guide. Given the value of using antibiotics and selectable antibiotic resistance markers for biomedical research, and the existing mechanisms already in place through other federal regulations to protect the public from agents in the research laboratory environment, we believe this revision would be counterproductive to the public health mission.

We appreciate the opportunity to comment on the proposed changes to the *NIH Guidelines*.

On behalf of the CDC IBC, User's Group, and Internal Select Agents Program,
Kathleen Keyes

Kathleen F. Keyes, MS, SM(NRCM), CBSP
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Richard J. Karalus, Ph.D.
Director of Microbiology

CUBRC

The opinions expressed in this memo are those of the author and are not necessarily the official position of CUBRC.

From: Rich Karalus [mailto:karalus@cubrc.org]
Sent: Thursday, April 09, 2009 3:50 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Proposed Revision to NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

After reading the proposed changes in the wording in the new guideline, it seems to me that, if taken literally, as the government and lawyers often do, all work that included the use of antibiotic resistance would be forbidden. The wording "the deliberate transfer of a drug resistance trait to microorganisms, if such acquisition could compromise the ability to treat or manage disease caused by that microorganism in human and veterinary medicine, or agriculture." Would forbid any research performed with any antibiotics approved for human or animal use, since, by introducing the resistance, you have compromised treatment of any disease by eliminating one drug. Therefore, even if twenty other therapies were available, the act of reducing treatment by just one drug would still compromise treatment, even if it was considered an extremely small risk. Whether that "compromise" is significant enough (or not) to warrant a restraint of that use is not taken into consideration by the current wording. Therefore taken literally (as the government and lawyers frequently do), this would, in effect, eliminate the use of antibiotic resistance as a tool. Since the ultimate judgment is left with institutional IBCs (where I feel it belongs), the wording should be changed to state " the deliberate transfer of a drug resistance trait to microorganisms, if such acquisition could **reasonably** compromise the ability to treat or disease caused by that microorganism…". The addition of that simple word would allow the IBCs to use good judgment, rather than having a literal translation forced upon them.
Thank you for considering my input,
Rich Karalus

Richard J. Karalus, Ph.D.

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June 18, 2009

Jacqueline Corrigan-Curay, J.D. M.D.
Executive Secretary, Recombinant DNA Activities
Office of Biotechnology ActivitiesActivities, National Institutes of Health,
6705 Rockledge Drive, Suite 750, MSC
7985, Bethesda, Maryland 20892–7985.

Dear Dr Corrigan-Curay,

At the June 4, 2009 Biohazard Control Committee (BCC) meeting, some of the proposed revisions to the NIH Guidelines were discussed. The BCC has used the guidance in the NIH document "Biosafety Considerations for Research with Lentiviral vectors" available at http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html since its publication in 2006. We are seeking clarification on whether the proposed changes will reflect this document.

Background: Myles Brown, MD, Chair of the BCC, received the enclosed email advertisement and shared it with committee members. To focus our discussion, we used the example of a commercially available construct for Lenti/SV40 containing large and small T antigen to work through the questions posed in the March 4, 2009 Federal Register.

Committee Responses to questions in Fed. Reg:

(a) What are the risks with the use of replication incompetent integrating vectors in the laboratory? For example, preclinical research with recombinant lentiviral vectos are generated using a step involving replication. At the lower doses typically used in laboratory experiments, are the risks to the laboratory worker of such nonreplicating, synthethic NA research sufficiently low as to warrant exemption from the NIH Guidelines?

Comment: If use of lentiviral vectors is considered exempt from the NIH Guidelines due to the low volumes used in laboratory experiments (compared to gene transfer doses), [comment a, above] then Biosafety Level 2 practices may not be required or enforced.

(b) Since the increased risk associated with human gene transfer is in part related to the administration of higher doses, should the exemption be limited to experiments involving the handling of low quantities or doses of NAs? What quantity would not be expected to pose a biosafety risk?

Oncogenesis in humans is thought to require disruption of multiple cell regulatory pathways. Given the efficiency of gene transfer by lentiviral vectors, experiments that involve transduction of dominantly acting oncogenes or shRNA for tumor suppressor genes should be carefully reviewed. Of particular concern are experiments involving viral oncogenes such as SV40 large T antigen which is able to target multiple pathways simultaneously. The addition of SV40 small t antigen further increases the concern
In summary, increased volume or titer of vector would increase risk. However, it is not possible to define the quantity not expected to pose a biosafety risk, since the definition of a dominant-acting oncogene is that the presence of a single copy in the cell prompts proliferation.

(c) Are there examples of nonreplicating synthetic NA research that should not be exempt due to greater potential risks (e.g. expression cassettes for oncogenes or toxins?)

Although non-replicating, once packaged into virions, lentiviral vectors encoding dominantly acting oncogenes or shRNAi for tumor suppressor genes should not be exempt. With the discovery of the Merkel cell carcinoma associated polyomavirus it is clear that viral oncogenes carried by these viruses are involved in human carcinogenesis. In particular lentiviral vectors encoding both SV40 large T and small T antigen need careful review.
Summary of discussion: The BCC voted to share our concerns with the NIH Office of Biotechnology re: appropriate guidance for research using lentiviral vectors with oncogenic constructs.

Chemically synthesized nucleic acids:

The BCC also reviewed he question of whether clinical trials involving chemically synthesized small interfering RNAs (siRNAs) should be reviewed under Appendix M of the NIH guidelines. FDA review of chemically synthesized siRNA as a drug seems adequate; applicable safety data is available from antisense studies.

Thank you for the opportunity to share our concerns.

Sincerely yours,

Myles Brown, MD
Chair, Biohazard Control Committee
Dana Farber Cancer Institute

Karen Byers, MS, RBP, CBSP
Biosafety Officer

Enclosure: advertisement for commercially available vectors with oncogenic inserts.

Byers, Karen B

From: ABM [ink@floorcover.info]
Sent: Wednesday, June 10, 2009 5:43 AM
To: Brown, Myles,M.D.
Subject: Immortalizing any primary cell efficiently and reliably

abm™

Antibodies Pre-Made siRNA Pre-Made Lentivirus Pre-Made Adenovirus
Gene Name Search Products

Dear Valuable Customer:

Long Live Cell Immortalization !

- Immortalize any cell
- Unlimited cell supply
- Save time and effort

Lentiviral vectors

Product	Description	Qty	Cat.No	Price($)*
Lenti/SV40 virus	Recombinant Lentivirus containing SV40 large and small T antigen (10^6 cfu/ml)	10ml	G203	675.00
Lenti-hTERT virus	Recombinant Lentivirus (sense) (10^6 cfu/ml)	10ml	G200	675.00
Lenti-Myc T58A Virus	Recombinant Myc T58A Lentivirus (10^6cfu/ml)	10ml	G217	675.00
Lenti-Ras V12 Virus	Recombinant Ras V12 Lentivirus (10^6cfu/ml)	10ml	G221	675.00
Lenti-p53 siRNA Virus	Recombinant p53 siRNA Lentivirus (10^6cfu/ml)	10ml	G219	675.00
Lenti-Rb siRNA Virus	Recombinant Rb siRNA Lentivirus (10^6cfu/ml)	10ml	G223	675.00

Adenoviral vectors

Product	Description	Qty	Cat.No	Price($)*
Adeno-SV40 virus	Recombinant Adenovirus containing SV40 large and small T antigen (10^6 cfu/ml)	250ul	G210	850.00

Adeno-hTERT	Recombinant Telomerase Adenovirus (10^6 cfu/ml)	250µl	G205	850.00

Retroviral vectors

Product	Description	Qty	Cat.No	Price($)*
Retro/SV40 virus	Recombinant Retrovirus containing SV40 large and small T antigen (10^6 cfu/ml)	10ml	G212	675.00
Retro-E1/hTERT virus	Recombinant Retrovirus (10^6 cfu/ml)	10ml	G207	675.00

*For for-profit organizations and corporations, the purchase price is 1.5 times the listing price.

Tel: (604) 247-2416, 1-866-757-2414 Fax: (604) 247-2414
Email: order@abmGood.com Website: www.abmGood.com

Andrew G. Braun, Sc.D
Director of Biological Safety

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The opinions expressed in this memo are those of the author and are not necessarily the official position of the Harvard Medical School.

HARVARD MEDICAL SCHOOL
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Dear OBA,

There is a logical problem with the proposed change in the NIH Guidelines. It greatly narrows the Guidelines' range. According to Section I-A, (Third Paragraph):

> "In accordance with this change in the scope of the *NIH Guidelines* the term "recombinant DNA molecules" will be replaced with "recombinant and synthetic nucleic acid molecules"

The replacement requires the encompassing of *both* recombinant molecules and synthetic molecules; not either one or the other. For instance in Appendix K-II-D the replacement leads to the following:

> **Appendix K-II-D.** Cultures of viable organisms containing ***recombinant and synthetic nucleic acid molecules*** shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment (emphasis added).

This problem appears hundreds of times in the proposed Guideline changes.

OBA's intent was clearly to add synthetic molecules to the existing Guidelines. Instead the change reduces the Guidelines' range to absurdity.

A quick, but cumbersome fix is to replace the word "and" with "and / or."

The opinions expressed here are solely those of the author and do not reflect any Harvard position.

Sincerely yours,

Andrew Braun

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COMMITTEE ON MICROBIOLOGICAL SAFETY

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1 May 2009

NIH OBA office
6705 Rockledge Drive
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Dear OBA,

Attached are my personal comments on proposed changes in the *NIH Guidelines for Research Involving Recombinant DNA Molecules,* discussed in the Federal Register: March 4, 2009 (Volume 74, Number 41) [Pages 9411-9421].

The suggestions, conclusions and opinions in the pages which follow are exclusively mine. They do not reflect the suggestions, conclusions or opinions of Harvard University, Harvard Medical School, its affiliated institutions or any Harvard Faculty Committees.

I appreciate the opportunity to comment on the proposals outlined in the Federal Register and look forward to reading further comments from the public and to follow the changes in the *Guidelines.*

Sincerely yours,

Andrew Braun

Discussion: Section I-A, and Section I-B (Last Paragraphs) There is a logical problem with OBA's change in the NIH Guidelines. It greatly narrows the Guidelines' range.

According to the Federal Register text (Page 9414, Federal Register, Vol. 74, No. 41/ Notices) Section I-A, and Section I-B (Last Paragraphs) requires the following:

> "In accordance with this change in the scope of the *NIH Guidelines* the term "recombinant DNA molecules" will be replaced with "recombinant and synthetic nucleic acid molecules"

The replacement requires ***both*** recombinant molecules and synthetic molecules; not either one or the other. For instance, in Appendix K-II-D the replacement leads to the following:

> **Appendix K-II-D.** Cultures of viable organisms containing ***recombinant and synthetic nucleic acid molecules*** shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment (emphasis added).

This problem appears more than one hundred times in the proposed Guideline.

OBA's intent was clearly to add synthetic molecules to the existing Guidelines. Instead the change reduces the Guidelines' range to absurdity.

A simple fix is to replace "recombinant DNA molecules" with "recombinant NA molecules" where "NA" means "nucleic acid." The definition of "NA" can be given in Section I-B

Section I-A. There is a problem with the OBA's proposed change in this Section.

The proposed Section is:

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

The problem is the incorrect use of "constructing." While the term is appropriate in the April 2002 *Guidelines*, the proposed wording implies the construction of synthetic nucleic acids are specified in the *Guidelines*. This implication is incorrect – the *Guidelines* do not and should not specify anything dealing with the chemical construction of synthetic nucleic acids.

Section I-A might instead be:

> **Section I-A. Purpose.** ''The purpose of the NIH Guidelines is to specify practices involving the recombination of nucleic acids, no matter what their origin and means of construction, and the handling of these novel nucleic acid structures. The Guidelines also specify practices for the handling of organisms containing such molecules.''

Section I-B. There is a problem with the OBA's proposed definition change in this Section.

The current Section is:

> **Section I-B. Definition.** ''In the context of the NIH Guidelines, recombinant DNA molecules are defined as either: (i) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.''

The OBA's proposed Section is:

> **Section I-B. Definition.** In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as: (i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell, (ii) synthetic nucleic acid molecules that are chemically, or by other means, synthesized or amplified nucleic acid molecules that may wholly or partially contain functional equivalents of nucleotides, or (iii) molecules that result from the replication of those described in (i) or (ii) above.''

The problem is a highly convoluted syntax making it nearly impossible to understand the definition. For instance the meaning of item ii, in the original Section I-B, has confused virologists and Biosafety Officers for decades.

A simpler Section I-B might be:

> **Section I-B-a. Definition.** "In the context of the NIH Guidelines DNA, RNA and synthetic nucleic acids are considered to be nucleic acids no matter what their origin.

Use of this definition may simplify the problem of dealing with "and/or" and other cumbersome phrases. The terms "nucleic acid" or "nucleic acids" can replace several other phrases while maintaining clarity.

> **Section I-B-b. Definition.** "In the context of the NIH Guidelines recombinant nucleic acids (NA) are defined as molecules constructed by joining nucleic acid segments, no matter what their origin, into novel molecules that can (i) replicate in a living cell or (ii) generate downstream molecules that can replicate in a living cell.

This definition may reduce or eliminate the need for:

> **Section III-E-1**. Recombinant and synthetic nucleic acid molecules containing no more than half of the genome. . . . (see page 10 for full text)-

Section II-A-3. There are problems with the new paragraphs proposed by OBA for this Section.

> "[New Paragraph] While the initial 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 a chimera in which the parent agent may not be obvious. In such cases, the risk assessment should involve 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 an analysis of the functional attributes of these sequences (e.g., sequence associated with virulence factors, transmissibility, etc.). It may be prudent to first consider the highest risk group classification of any agent sequence included in the chimera. Other factors to be considered include the percentage of the genome contributed by each of multiple parent agents, and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as predicted in the original host context.
>
> "[New Paragraph] The IBC must also be cognizant that the combination of certain sequences 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. A new biosafety risk may occur with a chimera 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.

First, the use of "chimera" and "parent agent" are concepts new to the *Guidelines*. Neither appears elsewhere in the *Guidelines*. These concepts have meanings which differ from person to person.

The term "parent strain" is used widely in the *Guidelines*. The meaning is clear – A wild type from which a genetically modified organism is generated.

In the *Guidelines* "agent" is used as short-hand for human etiological agents (bacteria, chlamydia, fungals, parasitics, viruses, rickettsia, and prions.). Toxins are not included.

Second, it should be possible to shorten the paragraphs without jargon but retaining their essence. For instance:

> "[New Paragraph] Genetically modified organisms containing two or more nucleic acid segments, no matter what their origin, may necessitate a complex risk analysis. Preliminary analysis should consider the risk associated with original source sequences taking into account their virulence, transmissibility, *etc*. Because there may be unanticipated consequences of multiple genetic modifications, the possibility of greater or lower hazard than expected must be considered. It may be necessary to test the final modified organisms with *in vitro* or *in vivo* studies under the initial assumption they have high hazard."

Section III-A-1. There are problems with the OBA's proposed Section.

Please read the comment at the end of this critique of Section III-A (page 7).

Current Section III-A-1

> **Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation;**
>
> "**Section III-A-1** 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."

OBA's proposed Section of III-A-1

> "**Section III-A-1** 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 (see Section V–B, Footnotes and References of Sections I– IV). *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*." (Emphasis added)

First, the proposed part is overly detailed. A short statement should be adequate.

However, this is a propitious moment to expand the paragraph to include the publication of a list of acceptable drug resistance genes. The list may mollify those worried about the loss of standard marker or selection genes.

> "**Section III-A-1** 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. RAC will publish and an annually update a list of acceptable drug resistant traits based on similar precedents. Approved drug resistance mutation in the list will not require approval by the NIH Director or further evaluation by the RAC. Investigators wishing to expand this list should call OBA to discuss the safety of their additions (301-496-9838). In establishing the original acceptable resistance traits and its additions RAC will consider the possibility a drug's resistant trait may weaken the treatment of infected humans, animals and plants throughout the world."

Second, the use of parenthetical remarks by OBA in the Guidelines is excessive. The remarks destroy any flow in the text and are often of no obvious value. A particularly egregious example is the remark in the original and OBA's proposed Section-III-A-1, above. It is "(see Section V–B, Footnotes and References of Sections I–IV)". When the harassed Investigator reaches this section, in a completely different Section, she encounters the following:

> **Section V-B.** Section III, *Experiments Covered by the NIH Guidelines*, describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*" (see Section IV-B-7-c-(1)). For cases falling under Sections III-A through III-E, *Experiments Covered by the NIH Guidelines*, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research" (see Section IV-B-2-b-(1), *Institutional Biosafety Committee*). The Institutional Biosafety Committee may refer specific cases to NIH/OBA as part of NIH/OBA's functions to "provide advice to all within and outside NIH" (see Section IV -C-3). NIH/OBA may request advice from the RAC as part of the RAC's responsibility for

"interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels" (see Section IV-C-1-b-(2)-(f), *Minor Actions*).

It is difficult to easily determine which part of this Footnote applies to Section III-A-1. It appears the intent is to remind the reader that the IBC is responsible to decide the containment level and suggests (see Section IV-B-b-(1)). Several other parenthetical suggested are also noted. To make matters worse there is no link back to where the Investigator started.

In future versions of the *Guidelines* it would be wise to retain a professional editor and/or grammarian with a scientific background.

General Comment about Section III-A

OBA should consider the possibility of deleting the Section III-A in its entirety. The section applies only to the deliberate transfer of a drug resistance trait to microorganisms. It differs from Section III-B solely in the requirement that the NIH Director approve the transfer.

As the RAC is fully capable of deciding whether these genes can be transferred safely and the fact the NIH Director has far more important things to do than approving this highly technical issue the section should be moved to Section III-B

Please see the General Comment about Section III-A, above.

Section III–B–2. There is a problem with the new Section.

Section III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation

> "**Section III–B–2**, [New Section] 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 approve prior to implementation of these changes. An experiment will only be considered equivalent if, as determined by NIH/OBA, there are no substantive differences in experimental design or pertinent information has not emerged since submission of the initial III-A-1 experiment that would impact on this biosafety or public health risks for the proposed experiments. If such a determination is made by NIH/OBA, these experiments will not require review and approval under Section III- A."

It is strange that this section is placed in Section III-B. The text pertains only to Section III-A. Why is it placed in Section III-B? The basic idea in Section III-B-2 is that adequate precedents of studies involving deliberate transfer of drug resistance do not have to be approved by the NIH Director.

Buy replacing the current and OBA's proposed versions of Section III-A-1 with that suggested earlier in this document (page 6) there is no need for the new Section III-B-2.

It may be desirable to retain Sections III-A and III-A-1 as place-markers. Section A-1-a can be used in the future when as yet unknown developments will require the NIH Director's involvement.

Thus:

Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, andNIH Director Approval Before Initiation (See Section IV-C-1-b-(1), Major Actions).

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

Experiments considered as Major Actions under the NIH Guidelines cannot be initiated without submission of relevant information on the proposed experiment to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), the publication of the proposal in the Federal Register for 15 days of comment, review by RAC, and specific approval by NIH. The containment conditions or stipulation requirements for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D, Major Actions Taken under the NIH Guidelines, which may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section III-A-1-a. Currently there are no Major Actions.

Section III-C. There are problems with OBA's proposed change in this Section.

The current Section is:

> **Section III-C1. "**For an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into 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)."

OBA's proposed Section is:

> **Section III-C-1.** ''For an experiment involving the deliberate transfer of recombinant or synthetic nucleic acids into 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).''

There are two problems:

1) One problem here and in other Sections and Appendices is the use of "human gene transfer." While the term is apt in the current *NIH Guidelines* version, it does not cover "non-coding" sections such as shRNA, antisense RNA, and other current and future structures. These structures are not genes in the conventional sense. They may cause harm in clinical settings. Electroporation and liposome methods can transfer small nucleic acids to living cells and animals without viral vectors.

2) The "or" in "recombinant or synthetic nucleic acids" ignores the possibility that some recombinant nucleic acids will contain both coding native and coding synthetic segments.

Possible solutions for **Section III-C-1.**

> 1) "Human gene transfer" can be replaced by "Clinical recombinant nucleic acid transfer."
>
> 2) "recombinant or synthetic nucleic acids" could be replaced by "recombinant nucleic acids."

"Human gene transfer" appears at least 55 times in the current *NIH Guidelines*.

Section III-E-1. There are problems with OBA's proposed change in this Section.

Current Section III-E-1

> **Section III-E. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation**
>
> **Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus**
>
> Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J, Footnotes and References of Sections I-IV]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

OBA's proposed Section III-E-1

> **Section III-E-1**. Recombinant and synthetic nucleic acid molecules containing no more than half of the genome of any one Risk Group 3 or 4 eukaryotic virus (all viruses from a single Family being considered identical [see Section V–J, Footnotes and References of Sections I–IV]) may be propagated and maintained in cells in tissue culture using BL1 containment (as defined in Appendix G) provided there is evidence that the resulting nucleic acid in these cells are not capable of producing a replication competent nucleic acid. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, Experiments Involving the Use of Infectious Animal or Plant DNA or RNA viruses or Defective Animal or Plant DNA or RNA viruses in the Presence of Helper Virus in Tissue Culture Systems should be used. The nucleic acids may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than one-half of a genome.

First, there is no reason or justification for this Section's new restriction to Risk Groups 3 and 4. Why is Risk Group 2 deleted?

Second, on what basis is the choice of half the genome enough to reduce the containment to BL1 while a RG4 virus with 51% of the genome requires using BL4 containment?

Third, in those viruses reduced from BL4 to BL1 containment are BL4 procedures still required?

Fourth, reduction in biosafety containment permitted by the Section is dependent on the virus' inability to replicate. Would it not be better to simply eliminate the <50% requirement and leave the decision of biosafety containment to replication competence?

Finally, the definition in Section I-B defines recombinant nucleic acids non-replicating recombinant nucleic acids. Thus non-replicating molecules are exempt from the *NIH Guidelines*. Thus Section III-E-1 is redundant and can be deleted from the *NIH Guidelines.*

"Parenthetically," Appendix B-V-1 is also redundant:

> **Appendix B-V-1. Murine Retroviral Vectors**
>
> Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Why only murine retroviral vectors? The phrase "used for human transfer" can mean many things, none accurate.

Better:

> **Appendix B-V-1. Murine Retroviral Vectors**
>
> Retroviral vectors to be used for clinical recombinant nucleic acid transfer experiments (less than 10 liters) that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Section III-F. There are a several problems with OBA's proposed changes in these sections.

Original NIH Guidelines:

> **"Section III-F** The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required.

OBA's proposed NIH Guidelines

> **"Section III-F** [New Section] The following recombinant and/or synthetic nucleic acids 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 CDC/ NIH Biosafety in Microbiological and Biomedical Laboratories Manual)."

Section III-F There is no obvious reason for the parenthetic statement. While BMBL is a valuable source of information, its citation is irrelevant in this context.

> **"Section III-F-1** [New Section] Synthetic nucleic acids that can not replicate, and that are not deliberately transferred into one or more human research participants (see Section III-C and Appendix M)."

Section III-F-1 is redundant and confusing. It states non-replicating synthetic nucleic acids are exempt. Section I-B already exempts non-replicating nucleic acids, both natural and synthetic. However, Section III-F-1 does not exempt non-replicating nucleic acids if they are transferred to humans in clinical studies. The exception is not mentioned in Section I-B. The inconsistency can be resolved by deleting III-F-1 and adding the exception of clinical studies to the definition in Section I-B. The parenthetic comment directs the reader to two locations in the *Guidelines* which are irrelevant in this context.

Section III-F-2

OBA's proposed NIH Guidelines

"**Section III-F-2.** [New Section] Recombinant or synthetic nucleic acids 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-2 <u>If the I-B-1 definition is accepted</u> this item will read:

> "**Section III-F-2.** Nucleic acids 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."

Original NIH Guidelines:

> **"Section III-F-2** "Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent."

OBA's proposed NIH Guidelines

> "**Section III-F-3** Recombinant or synthetic nucleic acids that consist solely of the exact nucleic acid sequence from a single source that exists contemporaneously in nature."

Section III-F-3 If the I-B-1 definition is accepted this item will read:

> "**Section III-F-3** Nucleic acids that consist solely of the exact nucleic acid sequence from a single source that exists contemporaneously in nature."

Original NIH Guidelines:

> "**Section III–F–6** 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-6 for other classes of experiments which are exempt from the *NIH Guidelines.*''

OBA's proposed NIH Guidelines (just renumbered)

> **"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*."

First, this section is seems to be in conflict with Section IV-C-1-b-(1)-c. Thus the citation to this section (in Section III-F-8) may be inappropriate. Section IV-C-1-b-(1)-(c) currently reads as:

> **Section IV-C. Responsibilities of the National Institutes of Health (NIH)**
>
> **Section IV-C-1. NIH Director**
>
> **Section IV-C-1-b. Specific Responsibilities**
>
> **Section IV-C-1-b-(1). Major Actions**
>
> **Section IV-C-1-b-(1)-(c).** Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

Section C requires a list that is somewhat more prescriptive. It restricts exemptions to known physiological processes while Section III-F-8 does not. Section III-F-8 requires advice from the RAC, appropriate notice and opportunity for public comment. Hence the link to Section IV-C-b-(1)-(c) seems to be inappropriate.

Second, there is no "Appendix C, exemptions under Section III-F-8" in the **Guidelines.**

Requests from OBA:

[OBA Request] "In arriving at the conclusion that non-replicating synthetic nucleic acids pose limited risks to the public or environment, the RAC considered different types of potential experiments involving a range of possible exposures (e.g., dose, route) and nucleic acids (e.g., positive strand RNA viruses, replication incompetent integrating vectors). For most research, the risks were considered sufficiently low so that little benefit was considered to be gained by increased oversight, which may hinder research. However, some questions remained. The public is encouraged to submit written comments on the following questions raised by this proposed modification to distinguish between laboratory and clinical research with replicating and non-replicating NA molecules.

"The public is encouraged to submit written comments on the following questions raised by this proposed modification to distinguish between laboratory and clinical research with replicating and non-replicating NA molecules.

(1) Is there a sufficient distinction between the risks of basic and preclinical research with replicating vs. non-replicating synthetic molecules to warrant the exemption?

(a) What are the risks with the use of replication incompetent integrating vectors in the laboratory? For example, preclinical research with recombinant lentiviral vectors is coved by the current *NIH Guidelines* because the vectors are generated using a step involving replication. At the lower doses typically used in laboratory experiments, are the risks to the laboratory worker of such non- replicating, synthetic NA research sufficiently low as to warrant exemption from the *NIH Guidelines*?

[Response: The Guidelines should separate the construction of, say, lentivirus vectors, from their final use. Construction may involve replication. However the product - the vector – is not capable of replication and should be exempt. Thus core laboratories generating lentivirus vectors for investigators in other laboratories are under the Guidelines. The recipients of the vector, if shown to be replication incompetent, are exempt from the Guidelines.

Another point of view. A wise person and friend feels infection and insertion by retroviral vectors cannot be exempt, no matter how low the titre and inability to replicate.]

(2) Since the increased risk associated with human gene transfer is in part related to the administration of higher doses, should the exemption be limited to experiments involving the handling of low quantities or doses of NAs? What quantity would not be expected to pose a biosafety risk?

[Response: Non-replicating nucleic acid molecules, no matter what their origin, should be thought of as potential toxins. The hazardous quantity depends on the means of administration. Injection to the liver or brain may have very different effects from muscle or skin. The amount administered is almost irrelevant.]

(3) Are there examples of non-replicating, synthetic NA research that should not be exempt due to greater potential risks (e.g., expression cassettes for oncogenes or toxins)?

[Response: Yes. Non-replicating nucleic acid molecules, no matter what their origin, should be thought of as potential toxins. Under certain conditions they may act as vaccines and may elicit undesirable results – for instance, autoimmune disease]

(4) For human gene transfer research, are there classes of non- replicating molecules that should be exempt due to lower potential risks (e.g., antisense RNA, RNAi, etc.)? If so, what criteria should be applied to determine such classes?

[Response: There is no reason to think non-replicating molecules are always harmless. Huge doses of non-replicating molecules can have global effects. Non-replicating nucleic acid molecules, no matter what their origin, should be thought of as potential toxins.]

John M. Hunt, Ph.D(ABMM)

Independent Consultant in Clinical and Public Health Microbiology

The opinions expressed in this memo are those of the author and are not necessarily the official position of the Independent Consultant in Clinical and Public Health Microbiology.

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892-7985

Subject: Response to request for comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register*, 9411-21

Dear Office of Biotechnology Activities,

I am writing as a former NIH/NCI-funded laboratorian, former IBC community member for the University of Minnesota, and as a current member of the American Society for Microbiology, to ask that you NOT revise Section III-A-1 of the NIH Guidelines with the restrictive revision proposed on pages 9420-9421 of the March 4, 2009, Federal Register, volume 74, Number 41. The risks that I see to NIH funded research are the following areas:

1. Overloading Institutional Biosafety Committees (IBCs) with review of the many standard experimental protocols used in microbiology, genetics, and molecular biology laboratories involving use of selectable markers of antibiotic resistance to demonstrate transfer of genetic information from one source to a living, propagable microorganism. This usage of transferable resistance is so common that the IBCs may well be required to review many more protocols in which it is a standard method, and overlook more sophisticated and potentially dangerous aspects of gene transfer. The IBC workload is already huge and their oversight responsibilities more complex. The cannot afford to be swamped with relatively safe (see comment 2. below) and accepted practice review on top of their workload.

2. Biosafety precautions are already in place in research laboratories. These are effective in preventing release of any antibiotic-resistant organisms into the environment where they might serve as potential pathogens for humans, animals or plants. The laboratory directors and principal investigators should be well aware of the risks to humans, including laboratory workers and patients, with particular susceptibilities to infection with resistant microorganisms, and are responsible for ensuring observance of the appropriate biosafety level precautions for working with the organisms involved.

3. The proposed revision appears to make hypothetical risk now a subject for discussion by the IBCs. The IBCs would, with the proposed revision in place, now be required to research and deal with the many threads of hypothetical risk to humans and the environment, which is time consuming,

delaying to the laboratory needing to move the research ahead, and propagative of an endless "but-what-if" effort that our NIH-funded researchers can ill afford as long as they observethe appropriate biosafety precautions noted in comment 2. above. Although the intent of the proposed revision might be well-meaning in terms of protecting patients and the environment, sufficient safeguards are in place to prevent both the microbiological risk and this distraction of the IBCs.

Thank you for your consideration of my comments.

With best regards,

John M. Hunt

John M. Hunt, Ph. D., D(ABMM)
Independent Consultant in Clinical and Public Health Microbiology
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May 04, 2009

Office of Biotechnology Activities
National Institute of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, Maryland 20892-7985

RE: **Comment on Federal Register Notice of March 4, 2009: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)**

Dear Sir or Madam:

Isis Pharmaceuticals, Inc. is a leading innovator in the discovery and development of novel synthetic antisense drugs. Since 1989, Isis has focused on the characterization, manufacture and clinical development of this therapeutic class of drug product. For these reasons, Isis is well-qualified to comment on the proposed action by the NIH to expand the scope of the NIH Guidelines to include research with synthetic nucleic acids.

We agree with the statement that the biosafety concerns of virulence, transmissibility, and pathogenecity are determined by the end product, not by the production technique. We also agree that low risk experiments (Section III-F) should be exempt from RAC oversight thereby striking a balance between safety and overregulation. These concepts are consistent with the new exemption, Section III-F-1, for the use of synthetic, non-replicating nucleic acids in non-clinical research. A distinction has been made for human research due to a concern of greater risk from transgene effects, risks of insertional mutagenesis, and immunological responses. However, we submit that based on the characteristics of antisense oligonucleotides and the available clinical development experience, there is no basis for these concerns and that the exemption for synthetic non-replicating oligonucleotides should be extended to clinical research as well. The relevant experience for each of these concerns is addressed below.

Antisense Oligonucleotides Can Not Exert Transgene Effect

Short, synthetic, non-replicating antisense oligonucleotides are dramatically different from genes for the following scientific reasons. Antisense oligonucleotides, at approximately 20-nucleotides in length and 7,000 Daltons are much smaller molecules compared to genes which are typically a million Daltons or more and are embedded in plasmids or viral vectors that are much larger. Antisense oligonucleotides are short, chemically synthesized, chemically modified, and chemically analyzed molecules. These antisense molecules therefore lack the elements necessary to make them functioning genes and do not integrate into the genome. By contrast, the larger DNA gene therapy molecules, whether created by recombinant or synthetic means, are designed to be integrated into genomes and be transcribed into RNA to result in a protein. Thus, those gene therapy molecules must have all the elements necessary to assure proper genomic insertion and performance. Large nucleic acid molecules used in gene transfer must be delivered in vectors, such as viruses, or in cationic liposomes. By contrast, antisense drugs are typically delivered in saline and do not employ viral vectors to facilitate incorporation or induce amplification. This utilization of a vector system to incorporate a large piece of DNA is one of the more salient features that distinguish gene therapy from antisense oligonucleotides.

Pharmacologic Effects of Antisense Drugs are Transient

While gene transfer is designed to result in prolonged effects, antisense oligonucleotides are designed to interact pharmacologically with RNA and not to alter permanently or semi-permanently the genotype of the cell. Like small molecule drugs, antisense oligonucleotides bind to a receptor, in this case mRNA. The pharmacologic consequence of antisense binding to its target is reduction in translation of protein. The pharmacologic effect of antisense drugs has been shown to be dependent on dose and concentration, much like traditional pharmacologic agents. The pharmacologic effects of antisense inhibitors are also known to be transient as the oligonucleotide is degraded by endogenous nucleases, and normal translation of the mRNA returns. Therefore, like small molecule drugs the interaction of an antisense oligonucleotide with its mRNA target is transient, predicted by its pharmacokinetic properties, and repeated administration is required to obtain a sustained effect.

Short Oligonucleotides Can Not Insert Into Genome

There is negligible risk of insertional mutagenesis by short synthetic non-replicating oligonucleotides because these molecules do not possess the regulatory elements necessary for genomic incorporation or amplification. Antisense oligonucleotides are typically based on single-stranded DNA oligonucleotides with or without additional

chemical modifications. Based on the short length and the single-stranded nature, these molecules can not be directly incorporated into the genome. The minimum size of a DNA for homologous recombination is reported to be 400 to 500 residues and requires double-stranded molecules (Bollag et al. 1989; Lai and Lien 1999). Thus, the short sequence of nucleotides utilized by antisense molecules does not provide sufficient genetic information for genomic insertion or incorporation.

The concerns for insertional mutagenesis are further reduced based on the development experience with numerous antisense oligonucleotides. These molecules have been uniformly negative in the standard battery of gene toxicology assays where cellular exposure is very high. Furthermore, there has been no suggestion of effects related to insertional mutagenesis from chronic toxicology or life-time bioassays performed to date.

Immunologic/Inflammatory Effects Do Not Require Dual Oversight

The concern for potential immunological response likely reflects the adverse response of some patients to the viral delivery vectors, such as adenoviral vectors, used for gene therapy. While proinflammatory effects have been associated with some short chemically-synthesized oligonucleotides, the nature of these effects are very different than those observed for viral vectors. The best characterized of the synthetic non-replicating oligonucleotides are antisense oligonucleotides that are largely composed of single-stranded DNA, rather than RNA. For the short DNA-based oligonucleotides, the sequence motifs associated with greater immunological response, such as the CpG oligonucleotides, are well understood, and are excluded from clinical research. Furthermore, most antisense oligonucleotides currently in development contain additional chemical modifications on the ribose 2′-position that further mitigate an inflammatory potential.

The position of ourselves and others on this issue is that an immune response is not unique to drugs with biosafety concerns. Antisense oligonucleotides, small molecule drugs, biologicals and vaccines all share this potential concern. The characterization of these effects on an individual compound basis is guided by standard FDA directed development guidance. Thus, this concern alone dose not argue for a differentiated RAC oversight of small, chemically-synthesized antisense oligonucleotides to any greater degree than it argues for differentiated oversight of small molecule drugs. In Isis clinical trials to date, over 3,200 patients have received subcutaneous or intravenous administration of antisense oligonucleotides with over 500 of these subjects being treated for more than 3 months (Kwoh 2007) and over 150 subjects treated for more than 6 months. The resultant clinical profile suggests that antisense oligonucleotides, like small molecule drugs, are already adequately governed in the U.S. by the current regulatory paradigm of oversight provided by the Food and Drug Administration.

Conclusion

The characteristics of antisense oligonucleotides outlined above provide support for exemption from the additional regulatory oversight outlined in the NIH Guidelines and suggest that this exemption be extended to clinical trials as well. The criteria to exclude this class is based on its negligible risk of transgene effects and insertional mutagenesis. Furthermore, risk of immunological response is not unique to synthetically derived DNA, and in the case of antisense oligonucleotides is under the vigilance of the FDA. It is for these reasons, that we propose the RAC exempt both basic and clinical research with antisense oligonucleotides from regulatory review by the RAC.

Sincerely,

Richard S. Geary, PhD
Senior Vice President, Development

Scott P. Henry, PhD, D.A.B.T.
Vice President, Preclinical Development

Joseph F. Johnston
Vice President, Regulatory Affairs

References

Bollag RJ, Waldman AS and Liskay RM (1989) Homologous recombination in mammalian cells. *Annu Rev Genet* 23:199-225.

Lai LW and Lien YH (1999) Homologous recombination based gene therapy. *Exp Nephrol* 7(1):11-14

Kwoh , TJ (2007)
An Overview of the Clinical Safety Experience of First- and Second-Generation Antisense Oligonucleotides, in *Antisense Drug Technology, Principles, strategies and applications* (Crooke ST ed) pp 365-399, Taylor & Francis Group, Boca Raton, FL

Karin D.E. Everett, Ph.D

PUBLIC COMMENT REGARDING Dept. HHS, NIH Office of Biotechnology Activities; Recombinant DNA research: Proposed Actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

Life on earth is a four billion year old, uncontrolled work-in-progress. The processes and reagents involved are unknown, as the victors ate the losers. The Acting Director of the Office of Science Policy repeatedly states that new regulations will provide biosafety from synthetic self-replicating DNA and RNA.

Some RNA is self-replicating.
Monty Python's DNA is self-replicating.
Crystals are self-replicating.
Amyloid prion protein fibers are self-replicating.
DNA per se is not self-replicating.

The appropriate response to the 2006 report Addressing Biosecurity Concerns Related to the Synthesis of Select Agents would be to state that synthetic DNA is not a synthetic Select Agent. Synthetic DNA is not a biohazard.

Any DNA sequence can be synthesized. So every DNA sequence will be affected by the Proposed Actions of the Acting Director.

The Directors proposal thus will apply to sequence analysis, to primer synthesis, to restriction mapping and cloning, to plasmid design. To antigen production. The planets most productive and rapidly moving field of discovery will be restricted, loaded with paperwork, and made vastly more expensive. The Proposed Actions will drive molecular research and discovery into countries not so burdened. The U. S. Brain Drain will increase. NIH grant proposals will be reduced in effectiveness.

This Proposal for Action should be rejected.

Sincerely,
Karin D. E. Everett, PhD

Personal

MERCK Research Laboratories

Ekopimo Ibia, M.D., M.P.H.
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May 1, 2009

Office of Biotechnology Activities
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RE: FR Notice March 4, 2009: Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

Merck & Co., Inc. is a leading worldwide human health products company. Through a combination of the best science and state-of-the-art medicine, Merck's Research and Development (R&D) pipeline has produced many important pharmaceutical products available today. These products have saved the lives of or improved the quality of life for millions of people globally.

Merck Research Laboratories (MRL), Merck's research division, is one of the leading biomedical research organizations. MRL tests many compounds as potential drug candidates through comprehensive, state-of-the-art R & D programs. Merck supports regulatory oversight of product development that is based on sound scientific principles and good medical judgment.

In the course of bringing Merck drug and biological product candidates through developmental testing, clinical trials and licensure, Merck scientists have acquired extensive experience with recombinant and synthetic nucleic acid products and their safe handling. We have utilized that experience to author the comments below.

General Comments

Merck welcomes this reassessment of the NIH Guidelines in the context of biosafety concerns regarding synthetic nucleic acids. Merck supports the position that biosafety concerns should be independent of whether the molecules under consideration were derived using synthetic chemical methods or were synthesized in a living host, but rather should reflect the inherent virulence, transmissibility, and pathogenicity of the resulting product.

There is a class of molecules consisting of short synthetic nucleic acids, including chemically synthesized siRNA, microRNA, antisense oligonucleotides, immunomodulating oligonucleotides (e.g. CpG oligonucleotides) and aptamers that pose a low biosafety risk.

As a group, these oligonucleotide therapeutics should be exempt under NIH Guidelines for both basic and clinical research. The basis of the justification for the proposed exemption includes that they:

1. are non-replicating
2. do not integrate into or otherwise modify permanently the host genome leading to heritable changes
3. are not gene therapy in that they do not have the potential to express a gene product
4. are not infective or transmissible
5. exert a transient, reversible effect

The development of oligonucleotide therapeutics is regulated at the Federal level, and there has been experience in thousands of subjects with this class of drugs without biosafety issues having been reported. Additional Recombinant DNA Advisory Committee or Institutional Biosafety Committee oversight is not necessary, and carries the potential to significantly impede medical research involving this promising class of drugs.

Specific Comments

In addition to the above general comments, in the attached table, we provide specific comments on sections of the FR Notice on the Proposed Actions Under the NIH Guidelines, including Merck's responses to the questions posed for comment. In the left column of the table, we reproduce the relevant portions of the FR Notice, and the right column carries our comments and suggested changes (bold/italicized type for added text). See Attachment for more details.

We appreciate the opportunity to share our comments with respect to the Proposed Actions Under the NIH Guidelines. For further information or questions, please contact me by phone 202 508 4567 or email ekopimo_ibia@merck.com.

Sincerely,

Ekopimo Ibia, MD, MPH
Director
Global Medical and Regulatory Policy

Attachment enclosed

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
FR Notice March 4, 2009
Recombinant DNA Research: Proposed Actions Under the *NIH Guidelines* for Research Involving Recombinant DNA Molecules (NIH Guidelines)

FR Notice Text	Merck Reviewer's Comment
SUMMARY: In 2006, the National Science Advisory Board for Biosecurity, an advisory committee to the Secretary of the Department of Health and Human Services, the NIH Director and all Federal entities that conduct/support life sciences research published a report entitled "Addressing Biosecurity Concerns Related to the Synthesis of Select Agents." The report included a recommendation that the United States Government (USG) "examine the language and implementation of current biosafety guidelines 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 USG adopted this recommendation and asked NIH to review the *NIH Guidelines for Research with Recombinant DNA* (*NIH Guidelines*) to evaluate whether these guidelines need to be revised to address biosafety concerns for research with synthetic DNA. With the advice of the NIH Recombinant DNA Advisory Committee (RAC), which is responsible for advising the NIH Director on all aspects of recombinant DNA technology, including revisions to the *NIH Guidelines*, the following proposed changes were developed. As outlined in more detail below, the proposed changes will expand the scope of the *NIH Guidelines* to specifically cover nucleic acid molecules made solely by synthetic means. The changes apply to basic laboratory research and clinical research. In addition, changes were made to clarify the criteria for determining whether an experiment to introduce drug resistance into a microorganism raises important public health issues such that it must be reviewed by the RAC and approved by the NIH Director. Finally, the proposed amendments speak to the appropriate level of review for recombinant or synthetic experiments involving more than half but less than two-thirds of the genome of certain viruses in tissue culture. These changes were prompted by an increased understanding of the biology of certain viruses that demonstrate there may be biosafety risks with certain viruses that contain less than two-thirds of the viral genome.	Merck applauds this reassessment of the *NIH Guidelines* regarding biosafety considerations for synthetic nucleic acids.
Background: 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 galvanized segments of the scientific community. It also has captured the attention of the general	We acknowledge that in addition to the potential to advance scientific and medical discovery, there is the potential for

public and policymakers, prompting far-reaching questions about the potential use of these techniques--including the synthesis of novel forms of life. These techniques promise to accelerate scientific discovery and have the potential to yield new therapeutics for disease. This same technology may lead to the modification of existing or the creation of new pathogens with unexpected and potentially dangerous characteristics. In 2004, the National Research Council (NRC) published a report that made an important contribution to the development of biosecurity policy for the biological sciences, "Biotechnology in the Age of Terrorism: Confronting the Dual Use Issue." While this report was not the first to recognize this problem, and indeed the U.S. Government (USG) had already initiated an examination of security issues in the biological sciences, the NRC report laid out a series of actions to improve biosecurity in life science research, one of which was the creation of an advisory body. The USG recognized the need for such an advisory body and formed the National Science Advisory Board for Biosecurity (NSABB) to advise the U.S. Government on strategies for minimizing the potential for misuse of information and technologies from life sciences research, taking into consideration both national security concerns and the needs of the research community. The NSABB, as it is chartered, differs somewhat from the panel proposed by the NRC report, but has aims similar to those envisioned by the NRC committee. At the NSABB's first meeting, the Secretary of Health and Human Services tasked the NSABB with identifying potential biosecurity concerns raised by the rapidly advancing ability to synthesize select agents (7 CFR part 331, 9 CFR part 121, and 42 CFR part 73) and other dangerous pathogens. In 2006, NSABB published a report entitled "Addressing Biosecurity Concerns Related to the Synthesis of Select Agents." 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 often educated in disciplines that do not routinely include formal training in biosafety, e.g., engineering. These researchers may be uncertain about when to consult an Institutional Biosafety Committee (IBC). The NSABB recommended to the Secretary of the Department of Health and Human Services that the language and implementation of current biosafety guidelines be examined to ensure that such guidelines and regulation provide adequate guidance for working with synthetically derived nucleic acids. This recommendation on the need for biosafety guidance was considered by the Executive Branch through a trans-Federal policy coordination process. The recommendation on the need for biosafety guidance was accepted by the U.S. Government with the understanding that implementation would be through modification of the *NIH Guidelines* as appropriate. The changes to the *NIH Guidelines* would then be cross-referenced in the Centers for Disease Control and	undesirable consequences of nucleic synthesis technology. Within the broad category of synthetic nucleic acids, there is a subset of small, non-replicating chemically-synthesized nucleic acids that pose no risk of generating new pathogens or modifying existing pathogens. These molecules (oligonucleotide therapeutics) that include siRNA, microRNA, antisense oligonucleotides, immunostimulatory oligonucleotides and aptamers do not have the potential for virulence, transmissibility or pathogenicity and thus they should be exempt under *NIH Guidelines* for both basic and clinical research. Such exemption would be consistent with Section III-F of the proposed revisions, which "exempts those synthetic nucleic acid constructs that do not pose a significant biosafety risk."

Prevention/NIH publication entitled: *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

The Recombinant DNA Advisory Committee (RAC) considered the applicability of the *NIH Guidelines* to the creation of, and experiments with synthetic nucleic acids (``synthetic biology'') and whether the *NIH Guidelines* adequately address the biosafety concerns that may arise from this research. The proposed revisions to the *NIH Guidelines* are intended to clarify the applicability of the *NIH Guidelines* to research with synthetic nucleic acids and provide principles and procedures for risk assessment and management of such research.

While the initial NSABB recommendation focused on synthetic genomics, which is the synthesis of nucleic acids using chemical or other methods that do not require traditional recombinant DNA techniques, it was recognized that this may only be the first step in a research proposal. The synthetic nucleic acid will then likely be placed in cells or organisms. As it is articulated in the NIH Guidelines, it is the manipulation of the recombinant nucleic acids that leads to different biosafety concerns. As such, the focus of any review of synthetic genomics from a biosafety perspective needs to address the biological experiments that will be carried out. Therefore, with respect to the *NIH Guidelines*, the task was to review the biosafety considerations of introducing these synthetic nucleic acids into biological systems.

Synthetic genomics utilizes different techniques than traditional recombinant methods of synthesis; however, the ultimate product may be the same. The biosafety considerations in most cases are related to the product being produced more than the technique used. In other words, the technique for creating sequences of nucleic acids is not determinative of virulence, transmissibility and pathogenicity of the product, which are key considerations in biosafety. There is no one to one correlation between increasing nucleic acid diversity and increasing risk of harm. Indeed, what has developed in nature involves complex and highly regulated sequences of nucleic acids in which there is often synergy between genes. Bringing together a number of genes or sequences from different sources may result in a nucleic acid sequence that is not functional in an organism. On the other hand, a single nucleic acid change which could be done by recombinant or synthetic means could lead to a significant enhancement in virulence. The focus of a biosafety analysis should be on the product with consideration of the source of the sequences. Synthetic techniques may result in a greater range of products than recombinant methods but the underlying challenge is the same: trying to understand how those disparate parts will act together. Ultimately a biological analysis of the end results will be required.

Under the current risk assessment framework of the *NIH Guidelines*, the starting point for any risk assessment begins with an assessment of the parent organism from which the sequence is derived. As discussed under Section II, Safety Considerations, synthetic techniques may enable the synthesis of more complex chimeras containing sequences from a number of different sources. This increasing complexity may make the task of determining the parent organism more challenging. This is addressed in proposed language that will be added to the risk assessment section of the *NIH Guidelines* (see proposed changes to Section II-A). Therefore, the changes proposed below treat the biosafety risks of experiments that use recombinant and synthetic techniques as equivalent. Also, although it was recognized that synthetic genetic manipulation techniques are not necessarily a very recent development, the integration of other fields (for example, chemistry and engineering) may lead to rapid development of yet unknown products that may raise new biosafety risks not anticipated. The risk management framework being presented herein is based on the current science and that which appears to be feasible in the foreseeable future. The amendments will broaden the scope of the *NIH Guidelines*, which currently cover research involving DNA molecules created via recombinant techniques (i.e., joining of DNA molecules), to encompass nucleic acids that are synthesized chemically or by other means without the use of recombinant technology. As amended, the *NIH Guidelines* will apply to all nucleic acids. This is accomplished through changes in Section I-A, Purpose and Section I-B, Definition of Recombinant DNA Molecules. The required level of review will be based on the risk of the experiment, i.e. the risk to the laboratory worker, the public and the environment. Low risk basic research involving non-replicating synthetic nucleic acids will be exempt from the *NIH Guidelines* and from review at the local level. High risk basic and clinical studies may be subject to review by the RAC and the NIH. To effect these changes, four sections of the *NIH Guidelines* will be revised. The title of the document will be changed to *NIH Guidelines* for Research Involving Recombinant and Synthetic Nucleic Acid Molecules and throughout the *NIH Guidelines* the term recombinant DNA will be changed to recombinant and synthetic nucleic acids. In addition to broadening the scope of the *NIH Guidelines* to encompass synthetic nucleic acids, included are proposed amendments to two other sections of the *NIH Guidelines*, Section III-A-1 and Section III-E-1, in order to (1) clarify the oversight of recombinant experiments involving the introduction of drug resistance traits and (2) to change the level of review for recombinant or synthetic experiments involving more than half but less than two-thirds of the genome of certain viruses in tissue culture. These proposed amendments were recommended by the RAC.	Extension of the *NIH Guidelines* to encompass all synthetic nucleic acids without consideration of the nature of the molecules is too broad. Oligonucleotide therapeutics present low biosafety risk, so as a class they should be exempt for not only basic research but also for clinical trials. Even in the setting of clinical studies, the risk of non-replicating oligonucleotide therapeutics to investigators, the public and the environment is sufficiently low to justify exemption.

Section III-A-1 requires certain experiments involving the transfer of drug resistance traits to microorganisms to be reviewed by the RAC and approved by the NIH Director. The current language has raised concerns from IBCs and investigators seeking to identify those experiments that require this heightened review. The revisions to Section III-A-1 will clarify that all experiments involving the transfer of a drug resistance trait to a microorganism will be subject to RAC review and NIH Director approval if the microorganism's acquisition of the trait could compromise public health. The changes will clarify that the microorganism's ability to acquire the trait naturally is not relevant to the safety of the experiment, that the provisions apply even if the drug at issue is not considered the ``drug of choice,'' and that adverse effects on population subgroups need to be considered. Under the *NIH Guidelines*, approval for an experiment under Section III-A is specific to the investigator submitting the proposal. Recognizing that this may not be an efficient use of resources and may slow important research, a new provision will authorize OBA to make a determination that a proposed experiment that would fall under Section III-A is equivalent to an experiment that has been reviewed previously as a Major Action and approved by NIH Director. In such cases, OBA will have the authority to permit this research to proceed without going through RAC review and NIH Director approval if OBA determines that there are no substantive differences in experimental design and pertinent information has not emerged since submission of the initial experiment that would impact on the biosafety or public health risks for the proposed experiments. Section III-E-1 of the *NIH Guidelines* currently states that tissue culture experiments involving viral constructs that contain less than two-thirds of the genome of any one of the high risk viruses may be performed at the lowest containment level (Biosafety Level 1) and initiated upon registration with the local institutional biosafety committee. The change proposed to this section will increase the threshold to less than one-half of the viral genome and require evidence that the resulting nucleic acid molecules are not capable of producing a replication competent virus. These changes are prompted by an increased understanding of the biology of certain viruses for which there may be biosafety risks for research involving less than two-thirds of the viral genome. These recommendations were adopted unanimously by the RAC at its March 2008 meeting. Included in these proposed changes are targeted questions that were considered in developing the proposed revisions to the *NIH Guidelines*. NIH requests not only comments on the proposed changes but also comment on the specific issues raised by these questions. It should be noted that the *NIH Guidelines* currently apply to research that is conducted at or sponsored by institutions that receive NIH funding for any research involving recombinant DNA.	

Due to these proposed changes, the *NIH Guidelines* will apply to research that is conducted at or sponsored by institutions that receive NIH funding for any research involving recombinant DNA and synthetic acid molecules. In addition, other, non-NIH, U.S. Government agencies, including the Department of Defense, the Department of Veterans Affairs and Department of 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 govern all Government funded research, it may be used as a tool for the entire research community to understand the potential biosafety implications of their research. In reviewing the proposed changes it is important to understand that *NIH Guidelines* outline appropriate biosafety practices and containment measures for laboratory recombinant DNA (rDNA) research and govern the conduct of clinical trials that involve the deliberate transfer of rDNA, or DNA or RNA derived from rDNA, into human research participants. The focus of the *NIH Guidelines* is on the risks to laboratory workers, the public and the environment associated with rDNA research and if implemented, synthetic nucleic acid research. The *NIH Guidelines* do promote the use of biological containment through the application of highly specific biological barriers that may limit the infectivity, dissemination, or survival of recombinant agents outside the laboratory. Biological containment may, therefore, mitigate the consequences of intentional misuse of such agents but does not directly address biosecurity issues raised by deliberate exposure outside of a research setting. As revised, the *NIH Guidelines* will continue to focus on the biosafety aspects of research with recombinant and synthetic nucleic acid molecules. There may also be biosecurity or dual use research concerns with some research involving recombinant or synthetic nucleic acid molecules, but that is beyond the scope of the *NIH Guidelines*. Biosecurity aspects of research involving infectious agents are addressed in other venues, including for example, in the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (Section VI, Principles of Laboratory Biosecurity) and the Select Agent Rules (42 CFR 73, 9 CFR part 121 and 7 CFR part 131). In addition, the U.S.G. continues to address these issues. For example, the NSABB is developing recommendations for the oversight of dual use research and is also addressing the issue of personnel reliability among individuals working with select agents.	
Proposed Amendments to the *NIH Guidelines* In order to ensure that biosafety considerations of synthetic biology research are addressed appropriately, the NIH is proposing the following changes to the *NIH Guidelines*:	
Title of the *NIH Guidelines* The title of the document is proposed to be changed from the *NIH Guidelines* for Research Involving Recombinant DNA Molecules to the *NIH Guidelines for Research Involving Recombinant*	

and Synthetic Nucleic Acid Molecules.	
Section I. Scope of the *NIH Guidelines* In order to clarify the applicability of the *NIH Guidelines* to research involving synthetic nucleic acids (NA), the following modifications are proposed to Section I, Scope of the *NIH Guidelines*.	
Section 1-A. Purpose Section I-A (Purpose) of the *NIH Guidelines* currently states that: ``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 is proposed to be amended to read: ``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, or (iii) organisms and viruses containing such molecules." As a result of these modifications, the *NIH Guidelines* will clearly apply to both recombinant and synthetically derived nucleic acids, including those that contain functional analogs of nucleotides (e.g., those used in artificially engineered genetic systems). In accordance with this change in the scope of the *NIH Guidelines* the term “recombinant DNA molecules” will be replaced with “recombinant and synthetic nucleic acid molecules.”	Please provide clarity on the meaning of "those wholly or partially containing functional equivalents of nucleotides" and of "functional analogs of nucleotides." Inclusion of illustrative examples of the equivalents or analogs under consideration would be very beneficial.
Section I-B. Definition of Recombinant and Synthetic Nucleic Acids The current definition of recombinant DNA molecule in the *NIH Guidelines* (Section I-B) is limited because it only explicitly refers to DNA and requires that segments be joined, which may not need to occur in research with synthetic NAs. The proposed revisions to the definition would retain a definition of recombinant NA similar to the current one for recombinant DNA but also add synthetic NA created without joining of segments. The current definition of recombinant DNA in Section I-B of the *NIH Guidelines* is articulated in three paragraphs labeled as A, B, and C in this notice only. Paragraph A states: “In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either: (i) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.” Paragraph B 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*.” Paragraph C 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 following modifications are proposed to Section I-B. Definition of Recombinant DNA Molecules: Paragraph A is proposed to be revised to read: ``In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as: (i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell, (ii) synthetic nucleic acid molecules that are chemically, or by other means, synthesized or amplified nucleic acid molecules that may wholly or partially contain functional equivalents of nucleotides, or (iii) molecules that result from the replication of those described in (i) or (ii) above." Paragraph B will no longer be included in the definition. It was added to the *NIH Guidelines* in 1982 to clarify that then novel synthetic DNA segments would be considered as equivalent to their natural DNA counterparts with regards to containment conditions; however, it only covered synthetic DNA if it produced a toxin or a pharmacologically active agent. The language presented difficulty in interpretation because of the lack of definition of ``toxin or a pharmacologically active agent." Paragraph B is proposed to be deleted due to the fact that the concepts are sufficiently covered in the following portions: The new (ii) in paragraph A which explicitly extends the scope of the *NIH Guidelines* to cover recombinant and synthetic constructs, and Section III-F (Exempt Experiments) of the *NIH Guidelines*, which as discussed later, exempts those synthetic nucleic acid constructs that do not pose a significant biosafety risk. Paragraph C will be deleted from this portion and will be moved to Section III-F of the *NIH Guidelines*. This is a proposed reorganization of the *NIH Guidelines* so that exempt molecules will be described in one place. A new Section IIIF-7 is proposed to read: ``Genomic DNA molecules of plants and bacteria that have acquired a transposable element provided the transposable element does not contain any recombinant or synthetic DNA" are not subject to the *NIH Guidelines*. In accordance with these changes in the scope and definition of the *NIH Guidelines*, the term ``recombinant DNA molecules" will be replaced with ``recombinant and synthetic nucleic molecules" throughout the *NIH Guidelines*.	We agree with exemption of synthetic nucleic acid constructs that do not pose a significant biosafety risk. Oligonucleotide therapeutics are synthetic nucleotide constructs that do not pose a significant biosafety risk, and their use for both basic and clinical research should be exempted.
Section III-C-1. Experiments Involving the Transfer of Recombinant DNA, or DNA or RNA Derived From Recombinant DNA, Into One or More Human Research Participants In accordance with the change to the scope and definition of recombinant DNA, the definition of human gene transfer experiments will be amended. The first paragraph of Section III-C-1 currently states: ``For an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into 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)." As	Given the proposed broadened definition of recombinant DNA that may be interpreted to include all synthetic nucleic acids, specific mention should be made that oligonucleotide therapeutics are not included under this section. The proposed text would read: "For an experiment involving the deliberate transfer of recombinant or synthetic nucleic

amended the first paragraph will state: ``For an experiment involving the deliberate transfer of recombinant or synthetic nucleic acids into 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)."	acids into human research participants (human gene transfer) ***exclusive of short synthetic oligonucleotide therapeutics (including, but not limited to, siRNA, miRNA, antisense oligonucleotides, immunostimulatory oligonucleotides, and aptamers)***, 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)." Introduction of oligonucleotide therapeutics into human participants does not constitute gene therapy (gene transfer).
Section III-F. Exempt Experiments Additional modifications are proposed to augment or clarify experiments that are exempt from the *NIH Guidelines*, those listed in Section III-F. The exemptions under Section III-F are designed to strike a balance between safety and overregulation. They exempt certain nucleic acid molecules from oversight by the *NIH Guidelines* because their introduction into a biological system is not expected to have a biosafety risk that requires review by an IBC or the introduction of these nucleic molecules into biological systems would be akin to processes that already occur in nature and hence determining proper biosafety practices would be evident by the characteristics of naturally occurring sequence and/or would be covered by other guidances. Is there a risk that these exemptions could inadvertently exempt an experiment that is deserving of IBC review? First, it is important to recognize that with the exception of the new proposed III-F-1 discussed below, the exemptions from the original *NIH Guidelines* have been preserved with minor modifications. While synthetic synthesis of nucleic acids will potentially raise new biosafety concerns the exemptions focus narrowly on a small set of products that should not raise biosafety concerns that warrant IBC review whether created by recombinant or synthetic means. To emphasize that research exempt from the *NIH Guidelines* will still have biosafety considerations and that other standards of biosafety may apply, a modification is proposed to the introductory language. 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 proposed to read: ``The following recombinant and/or synthetic nucleic acids 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	Merck agrees that certain nucleic acid products, such as oligonucleotide therapeutics, should not raise biosafety concerns and therefore, they should be exempt from NIH RAC and IBC review.

to such research (for example, the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Manual)."	
Section III-F-1 A new exemption under Section III-F-1 will exempt synthetic nucleic acids that cannot replicate from the *NIH Guidelines* unless they are used in human gene transfer (see Section III-C-1). This exemption is proposed so that the *NIH Guidelines* apply to synthetic NA 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 NA that can not replicate or were derived from molecules that can replicate. The biosafety risks of using such constructs in basic and preclinical research are believed to be 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 identical to that observed with chemical exposures, although nucleic acids are generally far less toxic than most chemicals. Members of the RAC Biosafety Working Group noted that one of the original impetuses for creating a special biosafety oversight for recombinant DNA research was the novel biosafety risks to the individual laboratory worker, the public health, and the environment presented by the ability of novel replicating nucleic acids to disseminate and persist within and outside of the laboratory. This risk of transmissibility is distinct from chemicals or other toxins, because of the potential for long-term persistence. Human gene transfer clinical trials should be differentiated from basic research. Current human gene transfer trials often involve non-replicating recombinant molecules. These are captured by the NIH Guidelines (see Section III-C-1 and Appendix M), because they are derived through recombinant technology that has steps involving replication (e.g., replication incompetent vectors, RNAi or antisense RNA expressed from vectors are all derived from replicating systems). The biosafety and health risks for human gene transfer for synthetic non-replicating nucleic acids are not fundamentally different from non-replicating recombinant vectors.	Merck supports the exemption of non-replicating synthetic nucleic acids for basic, non-clinical research as proposed in this section. For the purposes of human studies, the small, synthetic oligonucleotide therapeutics (e.g. siRNA, microRNA, immunostimulatory oligonucleotides) need to be distinguished from therapies delivered in plasmid or viral vectors (e.g. shRNA forms of RNAi, antisense RNA expressed in vivo, or gene therapy). We agree that such plasmid- and viral-based products should not be exempt from NIH Guidelines for human studies. Justification for exemption of oligonucleotide therapeutics for human studies include that they pose little risk to investigators, the public or the environment because they: • are non-replicating • do not integrate into or otherwise modify the host genome • are not gene therapy in that they do not have the potential to express a gene • are not infective or transmissible • exert a transient effect Also, antisense oligonucleotides have been administered to thousands of subjects in clinical trials without any evidence of biosafety issues. Adequate regulatory oversight of oligonucleotide therapeutics exists, and the addition of RAC and IBC oversight runs the risk of impeding valuable clinical research with little to be gained.

The safety distinction between laboratory research and human gene transfer is based on the difference in the potential health risk due to inadvertent lab exposure during basic or preclinical work and deliberate clinical gene transfer. The doses and routes of administration used in human gene transfer generally increase the risks. The 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, and immunological responses. For example, in the context of human gene transfer, the deliberate transfer of large numbers of replication incompetent retroviral vectors to hematopoietic stem cells in human clinical trials for X-Linked severe combined immunodeficiency disease contributed to the development of leukemia in some subjects starting several years after dosing. This is a unique situation in human trials that would not be replicated in a preclinical lab setting. Human gene transfer also raises scientific, medical, social and ethical considerations that warrant special attention and public discussion.	Merck supports continued RAC and IBC oversight of gene therapy vectors such as the retroviral vectors used as an example in this section, even if they are non-replicating. The risks regarding human gene transfer outlined in this paragraph (transgene effects and insertional mutagenesis) are not relevant to oligonucleotide therapeutics for the reasons outlined above in this response document. Also, although oligonucleotide therapeutics may induce immunological responses, and indeed in the case of oligonucleotide adjuvants are purposefully designed to do so, this is a feature that is shared with other small molecule drugs and with biologics that are not regarded to pose biosafety risks necessitating RAC or IBC review for human studies.
The following new exemption is proposed to be inserted as Section III-F-1; the current exemptions III-F-1 through III-F-5 are proposed to be re-numbered as III-F-2 through III-F-6. Section III-F-6 is proposed to become III-F-8, because a new section III-F-7 is proposed to be inserted. Section III-F-1 is proposed to read:	
Section III-F-1: Synthetic nucleic acids that can not replicate, and that are not deliberately transferred into one or more human research participants (see Section III-C and Appendix M).	For the reasons stated above, we propose that the exemption should read: "Section III-F-1: Synthetic nucleic acids that can not replicate, and that are not deliberately transferred into one or more human research participants***. Short, synthetic oligonucleotide therapeutics including siRNA, microRNA, antisense oligonucleotides, and immunostimulatory oligonucleotides are exempt for human research*** (see Section III-C and Appendix M)."
In arriving at the conclusion that non-replicating synthetic nucleic acids pose limited risks to the public or environment, the RAC considered different types of potential experiments involving a	

range of possible exposures (e.g., dose, route) and nucleic acids (e.g., positive strand RNA viruses, replication incompetent integrating vectors). For most research, the risks were considered sufficiently low so that little benefit was considered to be gained by increased oversight, which may hinder research. However, some questions remained. The public is encouraged to submit written comments on the following questions raised by this proposed modification to distinguish between laboratory and clinical research with replicating and non-replicating NA molecules.	
(1) Is there a sufficient distinction between the risks of basic and preclinical research with replicating vs. non-replicating synthetic molecules to warrant the exemption? (a) What are the risks with the use of replication incompetent integrating vectors in the laboratory? For example, preclinical research with recombinant lentiviral vectors is covered by the current *NIH Guidelines* because the vectors are generated using a step involving replication. At the lower doses typically used in laboratory experiments, are the risks to the laboratory worker of such non-replicating, synthetic NA research sufficiently low as to warrant exemption from the *NIH Guidelines*?	(1) Merck agrees that the distinction between replicating vs. non-replicating synthetic molecules is important in considering whether to exempt molecules or not. Merck supports exemption for basic and preclinical research of non-replicating oligonucleotide therapeutics that do not express a transgene and that exert their effect transiently for reasons outlined earlier in this document. (a) As highlighted in these questions the decision to exempt molecules cannot be made purely on the basis of replication competence. Other considerations may include the likelihood of reversion to replication competence and quantity of the materials being handled. It is hard to come up with a certain dose or quantity of experimental materials that should be exempt without considering a number of these variables on a case-by-case basis.
(2) Since the increased risk associated with human gene transfer is in part related to the administration of higher doses, should the exemption be limited to experiments involving the handling of low quantities or doses of NAs? What quantity would not be expected to pose a biosafety risk?	(2) Given different amounts of transgene expression due to a wide number of variables, it is difficult to prescribe a quantity of the gene therapy product that would be broadly exempted. Exemption would require a great deal of assurance that the gene therapy vector is non-replicating and not transmissible to any

	significant degree under conditions of use. While in some instances, there may be quantities that pose low risk, given this level of complexity, it is hard to envision criteria for exemption based on quantity alone for human gene transfer.
(3) Are there examples of non-replicating, synthetic NA research that should not be exempt due to greater potential risks (e.g., expression cassettes for oncogenes or toxins)?	(3) There are a significant number of non-integrating, transient vectors, and we would support their exemption, but for integrating vectors, we cannot support a blanket exemption. Accidental inoculation of a VSV-G pseudotyped lentiviral vector containing a transgene would not be inhibited from infecting corneal cells, integrating into the corneal cell genome and generating the transgene for a lifetime. Other possible concerns may result from insertion of a lentiviral vector, accidental activation of a silenced gene, or destruction of a transcribed gene. We believe that the IBCs need to retain oversight of such vectors, and should not be exempted from the guidelines. We fully support retaining oversight of both recombinant and synthetic expression cassettes which produce oncogenes or toxins. The risk assessment done by the IBC, as well as the ability to know the work is being performed on site and the subsequent ability to audit performance of the PI is invaluable.
(4) For human gene transfer research, are there classes of non-replicating molecules that should be exempt due to lower potential risks (e.g., antisense RNA, RNAi, etc.)? If so, what criteria should be applied to determine such classes?	(4) For the reasons outlined earlier in this response document, Merck proposes that chemically-synthesized oligonucleotide

	therapeutics that are 1) non-replicating, 2) do not lead to expression of a transgene, and 3) express their effect transiently, including, but not limited to, siRNA, microRNA, antisense oligonucleotides, immunostimulatory oligonucleotides and aptamers be exempt for human research studies. A distinction must be made from this oligonucleotide therapeutic class of drugs and the related therapies (e.g. shRNA and some antisense RNA) that are delivered by a plasmid or viral vector. Merck supports that such vector-based therapies that resemble gene therapy should remain under NIH RAC and IBC purview.
Section III-F-2 Section III-F-1 is proposed to be renumbered to III-F-2 and will be amended to clarify that replicating NAs that are not in cells (in addition to organisms and viruses) are exempt. Essentially, 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 extremely unlikely to have biosafety risks. The primary risks associated with all nucleic acids, whether synthetic or natural, are the effects these can engender when inside an organism or the cellular compartment. Nucleic acids can alter protein expression patterns in cells by binding to nucleic acids and blocking (1) replication of DNA, (2) transcription of DNA into RNA and (3) translation of RNA into protein. Furthermore, binding of synthetic or natural DNA to cellular nucleic acids may result in degradation of cellular DNA or RNA through the activity of natural cellular defense mechanisms. Natural or synthetic DNA may have catalytic activity (e.g., ribozymes) that can cleave target sequences in nucleic acids. It is these effects that can potentially lead the cell or organism containing the nucleic acid to pose a risk to laboratory workers, the public or environment. None of the effects described above will occur unless the nucleic acid is introduced into an organism, or a cell. Nucleic acids, by virtue of their physical and chemical properties do not readily penetrate cell membranes. The negative charge of a nucleic acid molecule effectively prevents transfer across the plasma membrane of a cell unless the negative charges of the molecule are either masked or neutralized by addition of chemical compounds (e.g., cationic lipids, calcium phosphate) or the cell membrane is physically perforated (e.g., electroporation) to enable penetration and uptake	Merck agrees that replicating NAs that are not in cells pose low biosafety risk and supports their exemption. The interference with DNA replication, transcription into RNA, or translation into protein and the degradation of cellular DNA or RNA are processes that may be a consequence or therapeutic effect of small molecule drugs not regulated by RAC or IBCs, and therefore do not represent a unique property of nucleic acids, nor do they by themselves represent a biosafety risk. The focus of this section should be restricted to the ability to replicate and thereby pose a risk to laboratory workers, the public, or the environment in the setting of introduction into a cell.

by the cell. In practice, the current *NIH Guidelines* cover the introduction or modification of recombinant DNA in tissue culture, organisms and viruses. Therefore, for clarity and in recognition that techniques have developed to more readily permit introduction of nucleic acids into cells, the amended F-1 speaks to cells, organisms and viruses. In addition, as stated above, natural barriers exist for entry of unmodified nucleic acids into cells. However, manipulation of molecules modified for improved penetration of cell membranes in the laboratory may have increased risk due to the enhanced ability to penetrate cell membranes and thus be able to replicate. Therefore, section III-F-1 is being modified to address such modified nucleic acids as well. Specifically, Section III-F-1 is proposed to be renumbered as III-F-2 and amended as follows: The current Section III-F-1 states: ``Those that are not in organisms or viruses."	
Section III-F-1 will be re-numbered to III-F-2 and is proposed to be amended to: ``Section III-F-2. Recombinant or synthetic nucleic acids 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."	To not include non-replicating molecules that pose low biosafety risk whether in delivery vehicles or not (e.g. non-replicating oligonucleotide therapeutics), Merck proposes that this section read: "Section III-F-2. Recombinant or synthetic nucleic acids 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. ***Also short, synthetic oligonucleotide therapeutics including siRNA, microRNA, antisense oligonucleotides, and immunomodulatory oligonucleotides, whether they are encapsulated in vehicles or not.***"
The proposed Sections III-F-3 through III-F-7 retain exemptions that were in the original *NIH Guidelines* with minor revisions. In reviewing these exemptions it is important to understand that it is not the goal of the *NIH Guidelines* to regulate all nucleic acid research but rather that subset of research that through recombinant or now synthetic means results in unique organisms or cells that potentially possess characteristics not yet seen in nature and hence pose potential safety risks both to	

the individual as well as the community should there be an inadvertent release. Specifically, the molecules that fall under the new Section III-F-3 (formerly Section III-F-2) are those that consist solely of the exact nucleic acid sequence from a single source that exists contemporaneously in nature. Those described in the new Sections F-4 and F-5 (formerly Sections F-3 and F-4) are nucleic acids that are being propagated in a host that is either the natural host for such nucleic acids or is a closely related prokaryotic or eukaryotic host. Again such constructs may already exist outside of a laboratory. Research that falls under F-6 (formerly Section F-5) is exempt because the manipulation of these nucleic acids in a laboratory setting would be equivalent to that which occurs in nature when certain organisms exchange genetic material via physiological processes (e.g., bacterial mating) outside of a laboratory setting. It is limited to those organisms that are already known to exchange DNA in nature. Finally, research that falls under the proposed Section F-7 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 in the *NIH Guidelines* of a recombinant DNA molecule. Unless a transposon has been modified to be a recombinant molecule, genomic DNA of either plants or bacteria that has acquired a transposon is not subject to the *NIH Guidelines*. This is because if these transposons have not been modified by the insertion of recombinant or synthetic DNA, they are equivalent to what is already in nature and the process occurs naturally outside of lab. The following changes are proposed for the Section III-F exemptions.	
Section III-F-3 Section III-F-2 is proposed to be re-numbered to III-F-3 and amended. In the current *NIH Guidelines*, research with molecules from a single DNA source is exempt. This would include molecules containing duplications or deletions; however, such molecules may present different risks than those of the wild type parent agents. The revised language is intended to clarify that exempt molecules must have the exact nucleic acid sequence from an organism that currently exists in nature in order to be exempt (e.g., because the 1918 influenza no longer exists in nature, research involving the reconstructed virus would not qualify for this exemption). The exemption does not imply that there are no biosafety risks associated with such research but rather recognizes that the *NIH Guidelines* do not apply to wild-type strains currently found in nature because a risk assessment for such work can be made with reference to the biological characteristics of the wild-type organism and are covered by other NIH biosafety standards (for example CDC/NIH Biosafety in microbiological and Biomedical Laboratories Manual). The following modifications are proposed for Section III-F-2. Section III-F-2 is proposed to be re-numbered to III-F-3 and amended as follows:	A strict reading of this section would appear to exempt a fully synthetic viral genome, such as the polio genome generated in 2002 (Cello, et al. Science 279, 1016, 2002), although such a synthesis may be covered under other (e.g. Select Agent) regulations. We would recommend that such molecules be handled at the containment level of the wild type parent agent, if the molecules are introduced into cells in which they are replication competent, or could replicate if accidentally introduced into a human.

The current III-F-2 states: "Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent." III-F-2 is proposed to be renumbered to III-F-3 and is proposed to be amended to state: "Recombinant or synthetic nucleic acids that consist solely of the exact nucleic acid sequence from a single source that exists contemporaneously in nature." This proposed modification would change "single nonchromosomal or viral source" to simply "single source." Specific comment is requested as to whether it is sufficiently clear that single source refers to "single chromosomal, non-chromosomal, or viral NA source" or should the language be specifically spelled out?	
Section III-F-4 The current Section III-F-3 is proposed to be renumbered to Section III-F-4 and amended. Section III-F-3 states: "Those that consist entirely of DNA 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." It is proposed to be amended as follows: "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 The current Section III-F-4 is proposed to be renumbered to Section III-F-5. Section III-F-4 currently states: "Those that consist entirely of DNA 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)." It is proposed to state the following: "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 The current Section III-F-5 is proposed to be renumbered to Section III-F-6. The current Section III-F-5 states: "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-5--Sublists of Natural Exchangers, for a list of natural exchangers that are exempt from the *NIH Guidelines*." It is proposed to be amended to state: "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 A1-through A-VI will be amended to reference Section III-F-6 rather than III-F-5.	
Section III-F-7 A new Section III-F-7 is proposed to be added. This proposed new Section takes an exemption that was previously included in the original definition (Section I-B) and moves it to this Section so that the definition of recombinant and nucleic acids found in the proposed Section I-B is solely a definition and does not include exemptions. The proposed 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. Section I-B: 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. New Section III-F-7 is proposed to state: Section III-F-7. Genomic DNA molecules of plants and bacteria that have acquired a transposable element provided the transposable element does not contain any recombinant or synthetic DNA.	
Section III-F-8 The current Section III-F-6 is proposed to be renumbered to Section III-F-8 and amended. This section provides a mechanism for the NIH Director to expand the exemptions to molecules not covered elsewhere in Section III-F. Research that falls under Section III-F-8 would need to have been reviewed and approved by the NIH Director following advice from the RAC and notice in the **Federal Register** to provide an opportunity for public comment. Only research that has been deemed to not present, following this extensive review process, a significant risk to health or the environment would fall under this section. Current Section III-F-6 states: "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-6 for other classes of experiments which are exempt from the *NIH Guidelines*." Section III-F-6 is proposed to be amended to state: "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 A1- through A-VI will be amended to reference Section III-F-8 rather than III-F-6.	
Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More Than Two-Thirds of the Genome of Any Eukaryotic Virus Experiments covered by Section III-E-1 can be initiated using Biosafety Level (BL) 1 containment simultaneously with Institutional Biosafety Committee notice. Section III-E-1 currently states: "Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [*see* Section V-J *Footnotes and References of Sections I-IV*]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA viruses or Defective Animal or Plant DNA or RNA viruses in the Presence of Helper Virus in Tissue Culture Systems*, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome." This section applies to viral constructs containing less than 2/3 of the genome of any virus (with all viruses from a single Family being considered as identical). However, concerns were raised that this level of oversight may not be adequate for research with potential synthetic biology agents derived from multiple segments of NA from a Family of viruses. In addition, some wild type viruses (e.g., herpes viruses) may be functional with less than 2/3 of the genome present. Therefore, the decision was made to propose to change 2/3 to one-half of the genome to reflect the current understanding of the biology of certain viruses. While the use of a quantitative measure to define properties of biological organisms is imperfect, the more conservative standard is consistent with Appendix C-1 *Recombinant DNA in Tissue Culture* which exempts from the *NIH Guidelines* recombinant DNA molecules from Risk Groups 1 and 2 that contain less than one-half of any eukaryotic viral genome. With this revision, experiments involving risk Group 3 and 4 viruses with less than one-half of any eukaryotic viral genome can be initiated at BL1 containment simultaneously with IBC registration provided evidence is also submitted attesting that the preparation(s) are free of replication competent virus, which may be generated through homologous recombination with endogenous proviruses or the use of a helper virus. If revised as proposed, an investigator will be permitted to initiate an experiment simultaneously with registration, since the retention of a quantitative standard provides such clear guidance. Section III-E-1 is proposed to be amended to state: "Recombinant and synthetic nucleic acid molecules containing no more than half of the genome of any one Risk Group 3 or 4 eukaryotic virus (all viruses from a single Family being considered identical [*see* Section V-J, *Footnotes and*	

References of Sections I-IV]) may be propagated and maintained in cells in tissue culture using BL1 containment (as defined in Appendix G) provided there is evidence that the resulting nucleic acid in these cells are not capable of producing a replication competent nucleic acid. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA viruses or Defective Animal or Plant DNA or RNA viruses in the Presence of Helper Virus in Tissue Culture Systems* should be used. The nucleic acids may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than one-half of a genome."	
Section IV-A Policy Section IV-A concerns the roles and responsibilities of the local institutions and investigators in implementing the *NIH Guidelines*. It contains a general policy statement that is often evoked as the "spirit'" of the *NIH Guidelines* because it acknowledges the inability of the document to describe specifically all conceivable research or emerging techniques; however, it remains the responsibility of researchers and institutions to adhere to "the intent of the *NIH Guidelines* as well as to their specifics." In order to emphasize that the *NIH Guidelines* are an evolving document which are expected to be modified to address new developments in research or scientific techniques, the following modifications are proposed to Section IV-A (Policy). 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 proposed to be amended to read: "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 experiments involving recombinant and/or synthetic nucleic acids cannot be foreseen. The utilization of new genetic manipulation techniques may enable work previously done by 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 DNA 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. In addition, as the field develops, new techniques and applications need to be monitored and assessed to determine whether revisions to the *NIH Guidelines* are needed. 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 recombinant and/or synthetic nucleic acids 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 and/or synthetic nucleic acid molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.”	
Section II. Safety Considerations Currently, the risk assessment framework of the *NIH Guidelines* uses the risk group of the parent organism as a starting point for determining the necessary containment level. For example, genetic modifications using 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 BL3 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* is applicable to experiments with synthetic nucleic acids. However, additional language is proposed to provide further guidance for evaluating research utilizing the capabilities of synthetic biology, as use of these techniques may lead to the creation of complex organisms for which identification of a parent organism, the starting point of the existing recombinant DNA risk assessment, is more difficult. Risk assessment may also be complicated by the limitations in predicting function from sequence(s) or the synergistic effects from combining	

sequences from different sources in a novel context.

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 regulation, 29 CFR 1910.1030, and OSHA publication 3127 (1996 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 three paragraphs are proposed to be amended by inserting the following two new paragraphs between the current first and second paragraphs of Section II-A-3:
"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*).

While the initial 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 a chimera in which the parent agent may not be obvious. In such cases, the risk assessment should involve 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 an analysis of the functional attributes of these sequences (e.g., sequence associated with virulence factors, transmissibility, etc.). It may be prudent to first consider the highest risk group classification of any agent sequence included in the chimera. Other factors to be considered include the percentage of the genome contributed by each of multiple parent agents, and the predicted

function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as predicted in the original host context. The IBC must also be cognizant that the combination of certain sequences 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. A new biosafety risk may occur with a chimera 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 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*."	
Section III-A-1. Major Actions Under the *NIH Guidelines* In reviewing the biosafety risks for synthetic genomics and biology and the different levels of review for each experiment, the RAC determined that it is important to also evaluate the class of experiments that require the highest level of review. In doing so, it was determined that the language for Section III-A-1 of the *NIH Guidelines* (research involving the introduction of drug resistance) does not clearly articulate the types of 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 multi-drug 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." Section III-A-1-a is proposed to be amended to: "The deliberate transfer of a drug resistance trait to	We believe the change in wording would have a significant, adverse effect on research. The inclusion of drugs not normally used to treat the organism used in the experiment will make it significantly more difficult for the IBC to determine which combinations of antibiotic resistance and organisms would require RAC review. The current wording has protected public health for over 30 years. We are not aware of any published evidence that increased antibiotic resistance results from the escape of a recombinant organism; rather it has been widespread, poorly controlled use in clinical and agricultural settings that have been the key drivers of this problem. This change would hamper useful research without

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 (see Section V-B, Footnotes and References of Sections I-IV). 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." The deletion of the phrase "that are not known to acquire the trait naturally" is proposed because the mechanism of acquisition should not be relevant as to whether these experiments pose potential public health risk and as such should receive a higher level of review. Moreover, all forms of antibiotic resistance occur naturally and the use of antibiotics creates selective pressure for resistant strains. The additional text recognizes that a drug may remain useful for control of a disease despite some percentage of the population of microorganisms having developed resistance. It is also intended to clarify that even if a particular drug is not considered the ``drug of choice" to treat a disease, elimination of such a drug as a treatment option may still raise important clinical and public health considerations for certain subpopulations. 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 III-B (Experiments that Require NIH/OBA and IBC Approval before Initiation) is proposed to be added to allow NIH/OBA the discretion to review and approve certain experiments if NIH/OBA determines that an equivalent experiment has already been approved by the NIH Director and there are no substantial changes to the proposed experiment or new information that would raise new biosafety or public health issues. Under this proposal, Investigators will be notified by NIH/OBA if such a determination has been made. The following addition is proposed to be added to Section III-B of the *NIH Guidelines* to allow NIH/OBA the discretion to review and approve certain experiments that have been previously reviewed by the RAC and approved by the NIH Director as a Major Action. "Section III-B-2, *Experiments that have been approved (under Section III-A-1-a) as Major Actions under the NIH Guidelines*	a significant improvement in public health.

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 in experimental design or pertinent information has not emerged since submission of the initial III-A-1 experiment that would impact on the biosafety or public health risks for the proposed experiments. If such a determination is made by NIH/OBA, these experiments will not require review and approval under Section III-A.”	

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Department of Microbiology

Midwestern University

The opinions expressed in this memo are those of the author and are not necessarily the official position of Midwestern University.

-----Original Message-----
From: Katzif, Sam [mailto:skatzi@midwestern.edu]
Sent: Thursday, April 23, 2009 3:22 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Comments regarding NIH Guideline revisions for Rec DNA molecules

OBA,

I support the comments submitted by The American Society for Microbiology (ASM) on April 9, 2009 regarding the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register, 9411-21. I request that the OBA consider these recommendations in lieu of the proposed changes in Section III-A-1.

Respectfully submitted,

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I disagree most strenuously with the decision by the OBA to revise the NIH Guidelines in Section III-A-1 Major Actions Under the NIH Guidelines.

This proposed revision states that all experiments involving “the deliberate transfer of a drug resistance trait to a microorganism, if such acquisition COULD [my emphasis] compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture,” will receive RAC review and NIH Director approval. This is in direct opposition to the current NIH Guidelines which specify that if the microorganism is known to acquire the trait naturally, then transfer of the drug resistance may not need RAC review.

The NIH is now proposing to delete the phrase “that are not known to acquire the trait naturally,” in Section III-A-1, based on no evidence of negative consequences requiring such a change. There has been to my knowledge no demonstration of a detrimental impact of the ability to treat or manage disease agents due to rDNA experimentation, after over 35 years of the use of drug resistance genes as selectable markers. Therefore, it should be the responsibility of the OBA to justify the need for this change in the guidelines before they are enacted.

Sincerely,

Joseph M. Cleary, PhD
Center Director
National Bioenergy Center
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401
303-384-6825
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Please note: The statements made by me here are my own views and opinion, and are not to be construed in any way to represent the policy of the US Department of Energy

Jonathan Visick, Ph.D
Department of Biology

North Central College

The opinions expressed in this memo are those of the author and are not necessarily the official position of the North Central College.

Dear Colleagues,

I am anxious to respond to the proposed changes to the NIH Guidelines Section III-A-1 involving introduction of drug-resistance traits into microorganisms.

I feel strongly that the changes as proposed will create major problems for investigators doing routine experiments in genetics, molecular biology and microbiology, as well as for educators--and without any offsetting positive effect on public health.

As a microbiologist, I am of course deeply concerned about the further spread of antibiotic resistance and maintaining our ability to combat bacterial disease. Further, I agree that what resistances might be acquired naturally is an outdated guideline that should be removed.

However, the guideline as proposed is likely to lead to the requirement that many if not all routine experiments using plasmid vectors containing common antibiotic resistance markers require review and specific approval. This would create an enormous regulatory burden that seems unlikely to protect public health. Despite more than 30 years of such experiments, there is no indication that the routine use of antibiotics and resistance genes in molecular biology has in any way impacted clinically important antibiotic resistance. I am in complete accord with the statement issued by the American Society for Microbiology on this matter.

Further, as an educator, I would add that this change would impact the training of young scientists, as well. While experiments conducted in teaching laboratories are not NIH-funded, it is common for institutions to apply NIH guidelines to experiments undertaken for educational purposes. Today, undergraduates routinely clone genes and transform bacteria with recombinant plasmids--indeed, many of the techniques now in common use in research-rich courses in genetics, molecular biology and microbiology rely heavily upon resistance markers. Implementation of
an over-strict standard could result in reduced quality of laboratory education at colleges and universities across the country.

Guidelines regulating the use of antibiotics and resistance genes are probably desirable; however, the proposed standard is too vague and would be too far-reaching if interpreted strictly. I strongly encourage you to seek further discussion with the scientific community prior to implementing any change to this section.

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Dr. Christina Eddy
Associate Professor Biology

North Greenville University

From: Chris Eddy [mailto:catfishergal1@yahoo.com]
Sent: Wednesday, April 22, 2009 1:37 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Federal Register notice: Volume 74, Number 41

I would like to comment on section III-A-1- Revising the criteria for determining when introduction of a drug resistance trait into a microorganism.

The changes proposed to the current regulations will significantly hinder research in microbial pathogenicity, physiology and genetics. The current language in the regulations regarding recombinant DNA techniques are sufficient to protect the public from harm.

Thank you for considering these comments.
Dr. Christina Eddy
Associate Professor Biology
North Greenville University

Franklin R. Leach
Professor emeritus, Biochemistry and Molecular Biology

Oklahoma State University

From: Leach, Franklin [mailto:franklin.leach@okstate.edu]
Sent: Wednesday, April 22, 2009 2:30 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: [FR Doc. E9–4618 Filed 3–3–09; 8:45 am]

The proposed change in the NIH rules us not needed to protect the public when the transfers permitted under the current regulation already occur in nature. This will have a chilling and inhibiting effect on research.

Franklin R. Leach
Professor emeritus, biochemistry and molecular biology
Oklahoma State University
Stillwater, OK

Oligonucleotide Safety Working Group (OSWG)

May 1, 2009

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, MD 20892-7985
Email: oba@od.nih.gov

Re: Comment on Federal Register Notice of March 4, 2009:
Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

The Oligonucleotide Safety Working Group (OSWG) is an assemblage of over 70 pharmaceutical professionals that actively address non-clinical issues and questions to facilitate the safe development of oligonucleotide drugs. The OSWG welcomes this update of the NIH Guidelines to account for the biosafety of synthetic nucleic acids.

Executive Summary

The OSWG believes that synthetic oligonucleotides that are not capable of being replicated, transcribed, or translated into proteins should be exempt under NIH Guidelines. This exemption should be not only for basic laboratory research, but also for human clinical trials. Short, chemically-synthesized oligonucleotides, hereafter in the document referred to as oligonucleotide drugs, are distinct from gene therapy, and thus do not share with gene therapy products the biosafety concerns raised in the March 4, 2009 Federal Register Notice. Because the oligonucleotide drugs exert a transient effect, cannot replicate *in vivo*, do not insert themselves into host genomes, are not capable of being copied into another nucleic acid or protein, and do not pose a risk of transmissibility, these molecules act more like classic small molecule drugs than like molecules used in gene therapy modalities. Therefore, oligonucleotide drugs should be differentiated from therapies that utilize vector-mediated technologies to introduce macromolecules carrying genetically encoded information that can be replicated, transcribed and/or translated to produce multiple copies derived from the initially encoded information in the drug molecule (collectively referred here as "functional genetic material"). Furthermore, oligonucleotide drugs are not perpetuated in bacteria or cell culture and hence have no potential to be contaminated with adventitious agents. Based on an understanding of the biologic activity of chemically-synthesized oligonucleotide drugs, the OSWG supports their exemption for basic laboratory research as proposed in the Federal Register Notice and supports their exemption also for human trials, given that the risks are sufficiently low that RAC review may hinder research and development, with little to be gained by the increased oversight.

Comments on the Proposed Actions Under the NIH Guidelines

In response to the RAC request for written comments on remaining questions, and based on the extensive experience of its members, the OSWG offers detailed remarks regarding small synthetic oligonucleotides to address concerns raised in the Federal Register Notice. These stated concerns include:

1. The method of creating sequences of nucleic acids does not determine virulence, transmissibility and pathogenicity, which are key biosafety considerations. (FR Notice pg. 9413, col. 1, par. 2)
2. The biosafety and health risks for human gene transfer trials for synthetic, non-replicating nucleic acids are not fundamentally different from non-replicating recombinant vectors. (FR Notice pg. 9415, col. 3, par. 4)
3. Nucleic acids can alter protein expression patterns by binding to nucleic acids and blocking DNA replication, transcription of DNA into RNA, or translation of RNA into protein. (FR Notice pg. 9416, col. 2, par. 6)
4. Modifications that improve penetration of cell membranes by nucleic acids may have increased risk due to the enhanced ability to replicate. (FR Notice pg. 9416, col. 3, par. 3)

.

Based on the following OSWG comments addressing these concerns, it is proposed that oligonucleotide drugs, whether associated with delivery vehicles or not, be listed as exempt in the NIH Guidelines for both basic laboratory and clinical research because they pose a low risk to investigators, the public, and the environment.

The definition of oligonucleotide drugs intended for this exemption would include short chemically-synthesized oligonucleotides that function via the antisense, siRNA, microRNA, ribozyme, splicing regulation, immunomodulatory or aptameric mechanisms of action.

Not included in this request for exemption would be plasmid- or vector-based expression systems intended to express an antisense or shRNA inhibitor.

The method of creating sequences of nucleic acids does not determine virulence, transmissibility and pathogenicity, which are key biosafety considerations.

Key to the biosafety of the oligonucleotide drugs is the fact that they cannot replicate, and that they cannot be transcribed or translated in cells. Also important is the notion that oligonucleotide drugs cannot replicate in microorganisms or in eukaryotic cells, so they do not pose a risk that persistence within and outside of the laboratory could lead to transmissibility. Given these characteristics, the potential concerns of synthesis of new forms of life or even mutant forms of existing agents with enhanced pathogenicity do not apply to oligonucleotide drugs.

Another concern raised in the Proposed Actions Under the NIH Guidelines relevant to potential pathogenicity and persistence is a potential for insertional mutagenesis. There have been no data signals suggestive of integration of antisense oligonucleotide drugs into cellular DNA. Chemically synthesized oligonucleotide drugs are typically 14 to 30 nucleotides long and do not possess the regulatory elements necessary for genomic incorporation or amplification. Single-stranded DNA oligonucleotides can not be directly incorporated into the genome. Double-stranded RNA oligonucleotides of 18 to 20-residues are distinct from double-stranded DNA and are too small to be incorporated into the genome through homologous recombination (Coffin, 1990). The minimum size of a DNA for homologous recombination is 400 to 500 residues (Bollag et al., 1989; Lai and Lien, 1999). Thus, the short sequences of oligonucleotide drugs do not provide sufficient genetic information to be inserted into the host genome.

Furthermore, in *in vitro* assays, vast numbers of cells are exposed to antisense drugs at near toxic concentrations. The cells are allowed to replicate multiple times over the course of these experiments, allowing amplification of any potential integration event. Despite the incubation conditions, these assays have been uniformly negative in both bacteria and mammalian cell lines, including human cell lines. These *in vitro* experiments represent vast numbers of potential integration events that are readily amplified if they occur; yet, the tests on dozens of sequences show no evidence of that. These data support the conclusion that antisense agents neither contain sufficient genetic information to be functional genes nor do they insert into the genome to produce heritable genetic changes or damage.

Recently compiled data from across the industry (as part of an OSWG initiative) indicate that dozens of oligonucleotide drugs have been negative in bacterial and mammalian genotoxicity assays. Lack of genotoxicity would suggest that there is a lack of integration occurring above the natural mutation frequency. Also, because of the uniform negativity in these genotoxicity assays, the European Medicine Agency no longer requires *in vitro* genetic toxicity testing of some antisense drugs (CHMP 2005).

In summary, oligonucleotide drugs pose little concern with regard to key biosafety considerations listed in the Federal Register Notice.

The biosafety and health risks for human gene transfer trials for synthetic, non-replicating nucleic acids are not fundamentally different from non-replicating recombinant vectors.

The OSWG supports RAC review for any synthetic or naturally occurring nucleic acids that are promoter- or vector-driven with the potential to express a transgene; however, we support the exemption for oligonucleotide drugs that do not have these characteristics. The OSWG also believes that synthetic oligonucleotides are fundamentally different from non-replicating recombinant vectors in two respects: 1)

they are not transcribed or translated and therefore do not enable the production of a plurality of copies of encoded mRNA and/or protein as would be expected for recombinant vectors and 2) they are not able to be propagated in cell culture systems that can introduce adventitious pathogens. The oligonucleotide drug itself is not a functional gene. It is critical to maintain the boundaries between oligonucleotide drugs that for the most part, act solely on the product of gene transcription or translation, RNA or protein, and those gene therapy agents that act directly to introduce genes or change the genome itself. Oligonucleotide drugs do not have regulatory sequences such as replicative origins, promoters, terminators and other elements required for generating a copy of itself, and oligonucleotide drugs are not capable of being translated into proteins. There is no mechanism for these drugs to self-propagate encoded information and amplify to generate multiple copies of itself like vector-driven therapies. Furthermore, oligonucleotide drugs are too small to be incorporated into the genome by homologous recombination. As such, oligonucleotide drugs are more like traditional drugs that are active as a result of exogenous administration, with the magnitude of effect determined by the administered dose and the concentration in the target organ. Oligonucleotide drugs then exert their effects by hybridizing to target RNA (antisense, siRNA, microRNA) or binding to target protein (aptamers, immunomodulating oligonucleotides), much the way low molecular weight drugs bind to their receptors. When the oligonucleotide drug is metabolized (by nucleases), the drug concentration decreases, and the pharmacological effect is diminished and ultimately reversed. This is in contrast to vector-driven gene therapy, where integration and amplification of a functional gene can permanently affect changes in target cells for a significant period of time and probably even result in gain of function effects in daughter cells or heritable changes. The activity of vector-driven gene therapy agents is dependent on cellular expression of the desired gene products, which is in turn dependent on the gene therapy agent using cellular machinery to produce an RNA or protein encoded by the transgene. Therefore, oligonucleotide drugs should remain distinct from therapies that use viral- or vector-mediated transformation of cells for the administration of functional genetic material.

Another concern raised in the Proposed Action under the NIH Guidelines in the context of human gene therapy was the potential to induce an immunological response. This concern is not unique to nucleic acids, being shared with drugs, biologics and vaccines that do not pose biosafety risks. Thus, the potential to induce an immune response should not lead to the classification of oligonucleotide drugs as gene therapy.

In summary, oligonucleotide drugs do not contain sufficient genetic information to be functional genes, nor do they insert into the genome to produce heritable genetic changes or damage. In more than 2500 subjects dosed with oligonucleotide drugs, no issues with biosafety have been reported (Kwoh 2008). Therefore, the OSWG proposes that oligonucleotide drugs be classified as exempt under NIH Guidelines for human clinical trials.

Nucleic acids can alter protein expression patterns by binding to nucleic acids and blocking DNA replication, transcription of DNA in to RNA, or translation of RNA into protein.

Alteration of endogenous protein expression by various means, including effects on DNA replication, RNA transcription and translation, may be shared by several approved or investigational drugs that currently are not regarded as posing a biosafety risk. Thus, rather than use this reasoning as a criterion for RAC review based on the ability to impact the stability or function of RNA, the OSWG recommends that oligonucleotide drugs should be exempt for basic and human studies based on the inability for replication or transmission, the transient duration of action without the potential for persistence due to insertion in the genome or heritability, and the inability to express a transgene.

Modifications that improve penetration of cell membranes by nucleic acids may have increased risk due to the enhanced ability to replicate.

While the medicinal utility of oligonucleotide drugs may be enhanced by improved delivery into cells, based on the fundamental properties outlined in the preceding sections, including their inability to replicate or to produce multiple copies of encoded information (RNA or protein), the low biosafety risk of oligonucleotide drugs cannot be affected by enhanced delivery. Just as other drugs administered with vehicles to enhance delivery to their targets (e.g. topical agents, and drugs in liposomal formulations or lipid complexes), the inability for transmission, replication and persistence of oligonucleotide drugs provide for their biosafety. Thus, the OSWG supports exemption of oligonucleotide drugs with or without the use of delivery vehicles.

Response to RAC Questions:

The public is encouraged to submit written comments on the following questions raised by this proposed modification to distinguish between laboratory and clinical research with replicating and non-replicating NA molecules.

(1) Is there a sufficient distinction between the risks of basic and preclinical research with replicating vs. non-replicating synthetic molecules to warrant the exemption?

OSWG Response: The scope of the OSWG includes only the non-replicating oligonucleotide drugs. As justified throughout this letter, the OSWG proposes that oligonucleotide drugs be exempt for basic, pre-clinical and clinical research.

(a) What are the risks with the use of replication incompetent integrating vectors in the laboratory? For example, preclinical research with recombinant lentiviral vectors is covered by the current *NIH Guidelines* because the vectors are generated using a step involving replication. At the lower doses typically used in laboratory

experiments, are the risks to the laboratory worker of such non-replicating, synthetic NA research sufficiently low as to warrant exemption from the *NIH Guidelines*?
OSWG Response: While we acknowledge the importance of this question, it is out of the scope for OSWG commenting.

(2) Since the increased risk associated with human gene transfer is in part related to the administration of higher doses, should the exemption be limited to experiments involving the handling of low quantities or doses of NAs? What quantity would not be expected to pose a biosafety risk?
OSWG Response: Like the previous question, this one is out of the scope of the OSWG because the oligonucleotide drugs are not gene therapy as summarized in this letter. However, as stated above, the OSWG does not include plasmid- or vector-based expression systems intended to express an antisense or shRNA inhibitor in its request for exemption of oligonucleotide drugs.

(3) Are there examples of non-replicating, synthetic NA research that should not be exempt due to greater potential risks (e.g., expression cassettes for oncogenes or toxins)?
OSWG Response: Because oligonucleotide drugs do not contain expression cassettes, the OSWG cannot comment on this question.

(4) For human gene transfer research, are there classes of non-replicating molecules that should be exempt due to lower potential risks (e.g., antisense RNA, RNAi, etc.)? If so, what criteria should be applied to determine such classes?
OSWG Response: This question is central to the content of this letter in which the OSWG has laid out the reasoning to support its proposal that oligonucleotide drugs be exempt from RAC and IBC review for both pre-clinical and human research. The OSWG has defined oligonucleotide drugs to include short chemically-synthesized oligonucleotides that act by antisense, siRNA, microRNA, splicing regulation, immunomodulatory or aptameric mechanisms of action. Five characteristics of these molecules that serve as criteria for exemption are enumerated in the following concluding section of this letter.

Final Comments

In conclusion, oligonucleotide drugs pose little biosafety risk because they:

1. do not replicate *in vivo*
2. do not pose a risk of persistence and transmissibility
3. do not contain functional genetic material
4. exert a transient effect, targeting RNA to degrade or inactivate it, or targeting protein
5. do not integrate into cellular DNA nor modify genomic sequences

To require RAC review of human trials using oligonucleotide drugs would run the risk of impeding the development of promising drugs for medical conditions that may be otherwise "undruggable" (i.e. molecular disease targets that would not be

amenable to small molecule or protein approaches) or may provide novel complementary approaches to current therapeutic modalities. Given the nature of oligonucleotide drugs, there is little scientific justification for addition of RAC oversight to the existing adequate regulatory supervision. Therefore, OSWG supports exemption under NIH Guidelines of oligonucleotide drugs for basic research and human clinical trials.

We appreciate the opportunity to share our comments with respect to the Proposed Actions under the NIH Guidelines.

For further information or questions, please contact me by phone at (617) 999-9422 or e-mail at schubertdh@logicaltherapeutics.com.

Sincerely,

David H. Schubert
Administrative Chairman
Oligonucleotide Safety Working Group

References

Bollag RJ, Waldman AS and Liskay RM (1989) Homologous recombination in mammalian cells. *Annu Rev Genet* **23**:199-225.

CHMP SWP Reflection Paper on the Assessment of the Genotoxic Potential of Antisense Oligonucleotides. European Medicines Agency Pre-authorisation Evaluation of Medicines for Human Use, London, 20 January 2005 (Doc. Ref.EMEA/CHMP/SWP/199726/2004)

Coffin JM (1990) Molecular mechanisms of nucleic acid integration. *J Med Virol* **31**(1):43-49.

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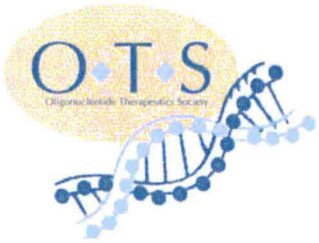
Lai LW and Lien YH (1999) Homologous recombination based gene therapy. *Exp Nephrol* **7**(1):11-14.

Oligonucleotide Therapeutic Society (OTS)



June 18th, 2009

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, MD 20892-7985
Email: oba@od.nih.gov

Re: Comment on Federal Register Notice of March 4, 2009:
Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

To Whom It May Concern:

The Oligonucleotide Therapeutic Society (OTS) is a non-profit organization whose mission is to foster academia and industry-based research and development of oligonucleotide therapeutics (RNAi, antisense DNA, ribozymes, CpG immunoadjuvants, and others). Founded in 2002, and with membership worldwide, OTS is proud to have become recognized as the premier organization devoted to fostering research and drug development in all areas of oligonucleotide science.

It is with the OTS's mission in mind that we, current and former Officers of OTS are writing to OBA. By this letter, we wish to go on record as being in complete agreement with our colleagues in the Oligonucleotide Study Working Group (OSWG), and the American Society for Gene and Cell Therapy (ASGCT), who have voiced concern over the RAC's intention to become involved in approving oligonucleotide drug trials. The reason the OTS is taking this position may be found in the contents of a letter written by David H. Schubert, Administrative Chair of the OSWG, and sent to OBA in response to Federal Register Notice of March 4, 2009: Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). Since we agree with the contents of this letter completely, and since we have the utmost respect for the OBA members' time, we will not reiterate the contents of this letter. Rather, we would only emphasize the following points about oligonucleotide based drugs:

1. Oligonucleotides do not contain genetic information which can be functionally expressed.
2. Oligonucleotides do not replicate *in vivo*
3. The physical structures, and effects, of oligonucleotides are transient by design.

4. There is no known, or in our view foreseeable, risk relating to the persistence or transmissibility of oligonucleotide drugs.

We are available for any questions you may have and hope to work constructively with you on this important issue.

With thanks in advance for your attention to our opinions on this matter, we are,

Sincerely yours,

Alan M. Gewirtz, MD
President- Oligonucleotide Therapeutics Society
C. Willard Robinson Professor
Division of Hematology/Oncology
Department of Medicine
University of Pennsylvania School of Medicine
Philadelphia, PA 19104

John G. Rossi, PhD
Immediate Past President- Oligonucleotide Therapeutics Society
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Professor, Department of Molecular Biology
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Mark A. Kay MD, PhD
Vice President- Oligonucleotide Therapeutics Society
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Departments of Pediatrics and Genetics
Stanford University School of Medicine
Stanford, California 94305

Pfizer

DNA Research: Proposed Actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

GENERAL COMMENTS ON TEXT		
Pfizer welcomes the opportunity to comment on the Federal Register Notice of 04 March 2009: Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)		
We propose that short synthetic oligonucleotides that can not replicate or be transmitted, should be differentiated from therapies where virulence, transmissibility and pathogenicity are of consideration, and should be listed as exempt under the NIH Guidelines for both basic laboratory research and human clinical trials.		
SPECIFIC COMMENTS ON TEXT		
Section/ Paragraph/line	Comment and Rationale	Proposal (if applicable) (proposed changes in verbiage in italics)
Section III-F1 Exempt Experiments:		
Page 9415	Short synthetic oligonucleotides including chemically synthesized siRNA, antisense oligonucleotides, microRNA, immunomodulatory oligonucleotides, ribozymes and aptemers are distinct from gene therapy and do not share these products biosafety concerns. Short synthetic oligonucleotides act transiently, can not replicate or be transmitted, pose low risk to investigators, the public and the environment and should be differentiated from synthetic nucleic acids where virulence, transmissibility and pathogenicity are key biosafety considerations.	It is proposed that oligonucleotide therapeutics be listed as exempt in the NIH guidelines for both basic laboratory and clinical research
Page 9415 Replicating versus non-replicating synthetic molecules	Synthetic oligonucleotides are not transcribed or translated, they do not generate mRNA or protein and are not propagable in cell culture systems that can introduce adventitious pathogens, they therefore differ fundamentally from non-replicating recombinant vectors	Oligonucleotide therapeutics should be considered distinct from therapies that use viral- or vector-mediated transformation of cells by the administration of functional genetic material and be considered exempt under the guidelines.
Page 9416 Risk of insertional mutagenesis	An extensive genotoxicity study of phosphorothioate ODN showed no evidence that phosphorothioate nucleotides pose a genotoxic risk (Henry, Monteith et al. 2002).	The uniform negativity of this data suggests a lack of integration above the natural mutation frequency, supporting the exemption of oligonucleotide therapeutics under the NIH guidelines
Page 9416 Risk of immunological responses	The potential to induce an immunological response is not unique to nucleic acids, being shared with drugs, biologics and vaccines that do not pose biosafety risks. .	The potential to induce an immune response should not prevent classification of oligonucleotide therapeutics as exempt under the NIH Guidelines for laboratory and human studies

SPECIFIC COMMENTS ON TEXT		
Section/ Paragraph/line	Comment and Rationale	Proposal (if applicable) (proposed changes in verbiage in italics)
Section III-F1 Exempt Experiments:		
Page 9416 Alteration of protein expression patterns	Alteration of endogenous protein expression by various means, including effects on DNA replication, RNA transcription and translation may be shared by several approved or investigational drugs that currently are not regarded as posing a biosafety risk.	The alteration of endogenous gene expression should not prevent classification of oligonucleotide therapies as exempt under the NIH guidelines. Oligonucleotide therapeutics should be exempt based on the inability for replication or transmission, the transient duration of action without the potential for persistence due to insertion in the genome or heritability, and the inability to express a transgene.
Page 9416 Manipulation of molecules modified for improved penetration of cell membranes	The inability for transmission, replication and persistence of oligonucleotide drugs cannot be affected by improved delivery into cells and provides for their biosafety	Oligonucleotide therapeutics with or without the use of delivery vehicles should be exempt under the NIH guidelines

Robert Reinhard

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May 30, 2009
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oba@od.nih.gov

RE: Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 74 Fed. Reg. 9411, March 4, 2009

To OBA:

Thank you for accepting comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register. I work with organizations, federal research trial networks and public/private partnerships investigating biomedical interventions to prevent and treat infectious disease, especially HIV/AIDS and other viral infections.

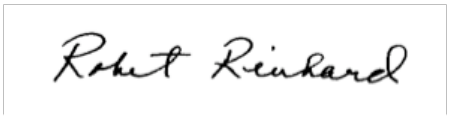
The proposed revised section states that experiments involving "the deliberate transfer of a drug resistance trait to a microorganism, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture," must receive RAC review and require NIH Director approval. The current NIH Guidelines state that if the microorganism is known to acquire the trait naturally, then transfer of the drug resistance may not need RAC review. The NIH proposes to delete the phrase "that are not known to acquire the trait naturally" in Section III-A-1.

I share the concerns of others such as the American Society for Microbiology (ASM) and the Association of American Medical Colleges (AAMC) on this proposed revision as it relates to concerns with antibiotics. I am also concerned with the inadvertent application of the proposed change to biomedical research involving naturally mutating viruses affecting either the host or transmission of resistant variants to oth-

ers. Without clarification, the wording of the appendix M guideline is so broad it could apply to a large number of research studies which already receive the necessary oversight by other agencies and review panels.

Along with others, I request that this proposed revision be withdrawn and that the Recombinant DNA Advisory Committee hold a public review of this proposed change, including potential effects on research for illness caused by viral pathogens.

Sincerely yours,



Robert Reinhard, consultant

Roche - Global Pharma Research

Roche

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, MD 20892-7985
USA

28 May 2009

Comment on Federal Register Notice of March 4, 2009: "Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)"

Dear Sir or Madam,

Roche is submitting this comment in response to the Federal Register Notice Vol. 74, No. 41 dated March 4, 2009 regarding the National Institutes of Health's Office of Biotechnology Activities: "Recombinant DNA Research: Proposed Action Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)," and the proposal to revise the guidelines to expand the scope to synthetic nucleic acid molecules.

Roche is one of the world's leading research-focused healthcare groups in the fields of pharmaceuticals and diagnostics. As the world's largest biotech company and an innovator of products and services for the early detection, prevention, diagnosis and treatment of diseases, Roche contributes across a broad range of the healthcare spectrum to improve people's health and quality of life.

Focusing on emerging technologies, Roche has made a substantial investment in new therapeutic platforms. In particular, Roche has established an RNA Therapeutics group to explore the potential of oligonucleotide based therapeutic modalities, specifically small interfering RNAs (siRNAs). Within Roche several preclinical programs using siRNAs are ongoing. As described in our attached comments, a significant amount of safety data has been generated with siRNAs, including data from non-human primates.

F. Hoffmann - La Roche AG

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Roche understands the need for the NIH initiative to update the scope of the NIH Guidelines to reflect scientific progress made since these guidelines were first established, and we commend the NIH RAC on this tremendous achievement. We are committed to supporting high safety standards with existing and future products. Roche is, therefore, very interested in contributing to the public discussion of the proposed revisions to the NIH Guidelines in order to ensure that public safety and the safety of individuals involved in basic research or in clinical trials involving siRNAs is not jeopardized and new innovative therapies become available to the public in a safe and efficient manner.

In formulating these comments, Roche has carefully considered:

1) the science and scientific data
2) the biosafety concern raised by the National Science Advisory Board for Biosecurity (NSABB)[1] which prompted the proposed changes
3) the questions posed by the Federal Register Notice.

Based on these considerations, and as outlined in detail in the attached discussion/rationale, Roche concludes that siRNAs are similar to other drugs such as small molecules, and as such, should be exempt from the NIH Guidelines.

Furthermore, we believe the FDA provides sufficient oversight to ensure safety to human subjects throughout the drug development process. Should the FDA require access to additional expertise, sufficient mechanisms exist for them to reach out to RAC members or other members of the academic community without mandating RAC oversight for siRNA-like molecules.

We appreciate the opportunity to share our comments with respect to the Proposed Actions under the NIH Guidelines.

Sincerely,

F. Hoffmann - La Roche AG

Lee Babiss, Global Head Pharma Research

Louis Renzetti, Global Head RNA Therapeutics

Thomas Singer, Global Head Non Clinical Safety

[1] to "examine the language and implementation of current biosafety guidelines 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"

Roche

Roche Comments on the Proposed Changes to NIH Guidelines for Research Involving Recombinant DNA Molecules

Introduction

Roche has studied the issues surrounding the proposed revisions to the NIH guidelines. In brief, Roche has concluded that: siRNAs and other oligonucleotide based therapeutic modalities, as outlined below, are similar to other drugs such as small molecules and, as such, should be exempt from the revision of the NIH Guidelines. In the process of arriving at these conclusions described in more detail below, Roche has evaluated 1) the Issues as discussed in the Federal Register (FR) notice of March 4, 2009: "Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules"; 2) the current NIH Guidelines; 3) the biosafety concerns raised by the National Science Advisory Board for Biosecurity (NSABB)[1]; 4) Roche's own experience with the RAC process; and 5) comments to the FR notice from other organizations (e.g. Oligo Safety Working Group, OSWG, and Alnylam). We agree with the need to include some classes of synthetic nucleic acids (NAs) under the scope of the NIH Guidelines and we agree with the comments presented by the OSWG and Alnylam.

Response to FR Notice Question #4

Regarding basic and pre-clinical research, the FR notice asks:

Question 4: For human gene transfer research, are there classes of non-replicating molecules that should be exempt due to lower potential risks (*e.g.*, antisense RNA, RNAi, etc.)? If so, what criteria should be applied to determine such classes?

Yes, Roche agrees that for human gene transfer research, there are classes of non-replicating molecules that should be exempt due to lower potential risks. Roche proposes, the exempt class should comprise siRNA[2].

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[1] To "examine the language and implementation of current biosafety guidelines 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"

[2] Also similar oligonucleotide drugs including short chemically-synthesized oligonucleotides with a miximalmaximal size of about 100 nucleotides that function via the antisense, microRNA, ribozyme, splicing regulation, immunomodulatory or aptameric mechanisms of action, should be exempt. This does not include plasmid- or vector-based expression systems intended to express an antisense or shRNAsiRNA inhibitor.

Roche's Rationale

In the oligonucleotide therapeutics community of which Roche is an active member, siRNAs are perceived as about 15-30 base pair long double-stranded RNAs that are chemically synthesized. The siRNA does not:

- function as a template for protein synthesis as it contains no mRNA elements (e.g. start codon and stop codon defining an open reading frame)
- integrate into the host genome
- modify genomic sequences
- replicate *in vivo* (Carl D. Novina and Phillip A. Sharp, The RNAi Revolution, *Nature* vol. 430 p. 161, 2004)
- pose a risk of persistence or transmissibility

As outlined in more detail in the comment of the OSWG of May 1, 2009 – that we fully support – as well as in our statement below, synthetic siRNAs lack all properties which pose a biosafety risk. Also, from a pharmacologic perspective, siRNA-based therapeutics are more closely related to traditional small molecule drugs than to recombinant nucleic acids. Therefore, we propose that siRNAs should not be categorized together with traditional recombinant DNA molecules, but rather be exempt from the proposed changes to the NIH guidelines.

Scientific Rationale

Fundamental difference between therapeutic approaches using synthetic siRNAs and vector-based approaches

It might have created a lot of confusion and uncertainty that, besides the use of synthetic siRNAs, there also exist vector-based gene-therapeutic approaches based on RNA interference (RNAi). In the case of a vector-driven gene therapy, integration and amplification of a functional gene can permanently affect changes in target cells for a significant period of time. We fully agree that this kind of gene-therapeutic use shall be ruled by recombinant nucleic acid guidelines. However, synthetic siRNAs are fundamentally different from recombinant vectors because they are not transcribed or translated and they are not able to be propagated. Therefore, siRNAs should remain distinct from therapies that use viral- or vector-mediated transformation of cells. Due to the fundamental differences it would be misleading and unwarranted to include synthetic siRNA in the scope of the guidelines. Roche is only using synthetic siRNAs, as outlined below.

Synthesis of siRNAs

The active pharmaceutical ingredient (API) of siRNA therapeutics consists of two short synthetic oligoribonucleotides that are complementary to each other and are typically 21 nucleotides long. The siRNA is designed such that one of the strands is complementary to a segment of a target messenger RNA (mRNA) that encodes a disease-related protein. For the synthesis of siRNA an automated solid phase chemical synthesis process is used where the oligonucleotides are built from synthetic synthones. This purely chemical process is scalable from small scale for screening purposes up to large scale required for GLP toxicology studies and clinical trials. Contract manufacturers are able to provide up to kg amounts of siRNA chemically synthesized under GMP conditions.

No cells or natural nucleic acids are involved in the whole siRNA production process, regardless of the scale.

Mechanism of Action

Within the cytoplasm of the cell, following unwinding of the two siRNA strands by an enzyme complex known as the RNA Induced Silencing Complex (RISC) one strand of the siRNA forms a temporary complex with RISC and scans the cellular mRNAs for a sequence that is complementary to the siRNA strand loaded in RISC. When a complementary mRNA strand is found, RISC cleaves the target mRNA at a defined location, and is then free to bind and cleave another mRNA. Cleavage leads to mRNA degradation which ultimately reduces the expression of the (disease-related) protein encoded by the target mRNA.

Neither the siRNA itself nor the RISC-bound strand of the siRNA interferes with DNA. Integration in or recombination with DNA is highly unlikely, if not impossible. There is no precedence for direct integration of an RNA molecule into genomic DNA. This would require the creation of a DNA copy of the siRNA which would be a highly complex process that is dependent upon certain enzymes and specific sequence elements. siRNAs are simply too short to provide such sequence elements. Thus, integration into the genome cannot be an unintended consequence of administering siRNA. The gene expression is reduced at the level of the mRNA. A great amount of *in vivo* data exists on the duration of effect with many different targets and in various animal species. In all known cases, the expression of the targeted gene returned to normal after a certain period of time following the last dosing with siRNA. In mice this

duration of effect is typically 2-4 weeks. No long-term effects were observed. With respect to any permanent reprogramming of target cells caused by a transient silencing of a target gene, siRNAs are not different from a small molecule or a therapeutic antibody, which transiently inactivate their target protein and thus may trigger longer lasting effects.

Delivery, Pharmacokinetics and Biodistribution

The major challenge for siRNA therapeutics is their delivery into the cytoplasm of the target cells. So far with each available delivery technology only a very small group of cell types could be targeted upon systemic administration of siRNAs. Those comprise, for example, hepatocytes that can be targeted by using liposomal formulations. By choosing a particular delivery vehicle, the target cell type can be selectively addressed. There is no evidence that delivery vehicles used, e.g., to achieve hepatocellular delivery, can target stem cells. Even if enhanced delivery can be achieved, the biosafety risk of siRNA drugs remains low given the fundamental properties of siRNAs, including their inability to replicate or to produce multiple copies in mammalian cells.

The concentration of the siRNA delivered to the cell determines the magnitude of the response. As outlined above, the pharmacologic response reverts to normal after a certain period of time as the siRNA is cleared like typical drugs as a result of metabolism. Unattended delivery to other cell types is rather unlikely. In particular, there is no evidence that stem cells are inadvertently targeted.

Pharmacodynamics

In contrast to recombinant nucleic acids that can be maintained in the cell, siRNAs are degraded by normal metabolism or excreted. In this respect there is no difference to small molecule drugs and protein-based therapeutics.

Safety

The concern exists that siRNAs can induce an immunological response. In fact, it could be shown that certain siRNAs stimulate the innate immunity through the activation of toll-like receptors. However, siRNA-mediated stimulation of innate immunity can be well controlled by using chemically modified siRNAs. Generally, the potential to stimulate an immunological response is not unique to nucleic acids. It is shared with drugs, biologics and vaccines that do not pose undue biosafety risks. Thus, the potential to induce an immune response by itself should not lead to the classification of siRNAs as gene therapy.

There is also the concern that siRNAs could target undesired off-targets. Off-targeting is assessed, evaluated and eliminated during the lead identification process on two levels. Due to the availability of the human transcriptome sequences, off-targets can be predicted using bioinformatics methods. siRNAs that have a certain potential to address off-targets are routinely precluded from the lead identification process. On the second level the best predicted off-targets are physically made and tested in the laboratory on their potential to be silenced by our siRNA lead candidates. If this is the case the respective siRNA candidate will be dropped. Given these precautions, the risk for off-targeting by siRNAs is not higher than with any other type of drug including small molecules which have the potential to target other proteins.

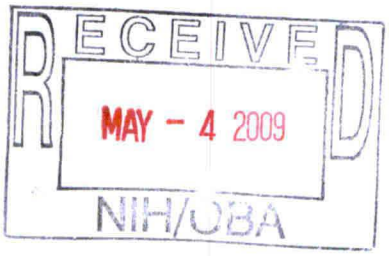
Since not all targets are expressed in all cell types, an additional layer of safety comes into play because the obligatory use of a delivery vehicle narrows down the number of target cell types and therewith the number of real off-targets.

Conclusion

With respect to all safety-relevant properties siRNAs are, as outlined above, similar to other drugs such as small molecules but dissimilar to recombinant nucleic acids and gene therapeutic approaches. We therefore conclude they should be exempt from the recombinant nucleic acid guideline and not require RAC oversight for proposed nonclinical or clinical studies.

Rowan University

Rowan University



23 April 2009

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892-7985

Subject: Response to request for comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 Federal Register, 9411-21

We, the undersigned faculty and staff at Rowan University, would like to state that we concur with the American Society for Microbiology's (ASM) response (dated 9 April 2009) to these proposed revisions. The proposed revision to Section III-A-1 is far too broad, and would be an impediment to basic microbial genetics procedures as described in the ASM response. If enacted, the result of the new Section III-A-1 regulation would be problematic enough at major research institutions, but it would be particularly burdensome at relatively small, primarily undergraduate institutions such as Rowan University.

As outlined by the ASM response, it is not entirely clear why the old Section III-A-1 guidelines are insufficient or why the new guidelines should be so broad. If there is strong evidence that this sort of concern is warranted, we would suggest that instead of limiting the kinds of experiments utilizing antibiotic resistance it might be more practical – and just as effective – to focus on proper containment and disposal of antibiotic resistant organisms.

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We thank the NIH for the opportunity to comment on the proposed guideline changes prior to their implementation.

Sincerely,

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Gregory A. Caputo
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Patricia J. Baynham, Ph.D
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St. Edward's University

The opinions expressed in this memo are those of the author and are not necessarily the official position of St. Edward's University.

From: Patricia Baynham [mailto:patricib@stedwards.edu]
Sent: Wednesday, April 22, 2009 2:13 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Proposed Action on RDNA Experiments Involving Drug Resistant Traits

Dear NIH OBA:

I am opposed to the following revision:

- Section III-A-1- Revising the criteria for determining when introduction of a drug resistance trait into a microorganism must be reviewed and approved by the NIH Director. NIH proposes to remove the current language regarding a microorganism's ability to acquire the trait naturally, stating that this criterion may not be determinative of the safety and public health implications of the research. As proposed, this portion of the NIH Guidelines would state, "the deliberate transfer of a drug resistance trait to microorganisms, if such acquisition could compromise the ability to treat or manage disease caused by that microorganism in human and veterinary medicine, or agriculture." The proposed amendment also contains additional language requiring consideration of the utility of the drug in certain subpopulations.

Microbiologists are extremely careful that experimental bacteria remain in the laboratory and there is no documented case of these bacteria becoming a public health threat. On the other hand the use of antibiotic resistance markers is necessary for much of the bacteriological research carried on across the USA and the world. Placing undue restrictions on the use of these markers in laboratory bacteria will hinder scientific research and the search for treatments and cures for diseases caused by bacteria. Additionally, these restrictions would put the US at a disadvantage with regard to scientific research and advances.

Sincerely,
Trish Baynham

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R. Kip Guy
Chairman, Department of Chemical Biology and Therapeutics

St. Jude Children's Research Hospital

The opinions expressed in this memo are those of the author and are not necessarily the official position of St. Jude Children's Research Hospital.

RAC Executive Secretary

I am writing to provide feedback on the proposed changes to the guidelines.

The proposed guidelines are much too broad and will provide an undue burden upon research institutions to provide regulation of the use of materials for which there is no clear gain in risk mitigation for public health. In addition, they may greatly hinder research into overcoming drug resistance which is clearly not in the public interest.

The guidelines need to be clarified to more clearly exclude commonly used reagents with minimal risk (for example oligonucleotides used in sequencing), and to restrict restrictions on drug resistant strains to cases where there is potential for public release of the materials.

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David Hunnicutt
Assistant Professor of Biology

St. Norbert College

-----Original Message-----
From: David Hunnicutt [mailto:David.Hunnicutt@snc.edu]
Sent: Thursday, April 23, 2009 9:11 AM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Proposed RDNA guideline change

To Whom It May Concern:

I echo ASM's concern over the changes in Section III-A-1 of the NIH RDNA guidelines. Bacterial genetic studies are virtually impossible without the use of selective markers. Selective markers are virtually all antibiotic resistance genes. The proposed changes would require review for thousands if not millions of experiments that have been conducted routinely for decades with no evidence of harm to the community. Indeed, the genes most commonly used as selective markers provide resistance to antibiotics not in frontline clinical use precisely because the resistance genes are so commonly found in natural isolates. Any additional risk associated with the transfer of these genes to low-virulence strains carefully contained in laboratory conditions must be vanishingly small. The effect of requiring NIH review for nearly every protocol in bacterial genetics, general DNA cloning, and a host of other common experiments conducted in research and teaching labs on a daily basis, however, is huge.

I respectfully ask the committee to reconsider this change.

Sincerely,

--
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Patrick Krieger
Veterinary Biologics Section

The Animal Health Institute (AHI)

The opinions expressed in this memo are those of the author and are not necessarily the official position of The Animal Health Institute (AHI).

The Animal Health Institute ("AHI") submits these comments to the Federal Register Notice "Office of Biotechnology Activities; Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)". AHI is the national trade association representing manufacturers of animal health products -- the pharmaceuticals, vaccines and feed additives used in modern food production, and the medicines that keep livestock and pets healthy. Our licensed member companies represent approximately ninety-five percent (95%) of the U.S domestic market for veterinary biological products, as well as serving a significant segment of the world market. As such, we have a tremendous interest in issues that affect veterinary biologics.

While these guidelines do not directly apply to APHIS-regulated biologic firms, the proposed amendments to the NIH guidelines could have a direct impact on APHIS-regulated firms since the Department of Agriculture references the NIH Guidelines when assessing recombinant DNA research. Consequently, these amended guidelines may establish a pivotal basis for the assessment and regulation of recombinant biological products.

As understood §IIIA1 — "Major Actions Under the NIH Guidelines" — a company is required to obtain approval of the RAC and NIH Director if introduction of a drug resistance trait "could compromise public health." This statement is vague. This section should clarify and narrowly focus on the areas of concern. It would also be beneficial to also include a list of criteria that could be considered "compromising public health" that can be used by an Institutional Biosafety Committee (IBC) during a risk assessment. This could also include examples of genes that are considered generally safe — such as kanamycin resistance, which has been approved as a safe food additive by the FDA.

We believe there is a need to develop clear guidelines that provide direction on how resistance markers can and will be assessed using science-based risk criterion. This should take into consideration that levels of risk can be greatly mitigated by key application methods and carefully designed use provisions. These guidelines should be developed through joint collaboration between the NIH, the Recombinant DNA Advisory Committee (RAC), representative of the Office of Biotechnology Activities and affected academic/industry groups. Without mutual concurrence, we are concerned that any guideline prohibitions based on possibility scenarios with extremely low probabilities of occurrence could have a profound effect on current and future medicinal product availabilities.

We appreciate the opportunity to comment on NIH guidelines. Should there be a need for further dialog or clarification, please do not hesitate to contact us.

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Topigen Pharmaceuticals

April 28, 2009

TOPIGEN

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Email: oba@od.nih.gov

Re: Comment on Federal Register Notice of March 4, 2009: Recombinant DNA Research: Proposed Actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules

Executive Summary

Topigen Pharmaceuticals, a Montreal based drug company which is currently developing oligonucleotide products as innovative therapeutics for respiratory diseases, welcomes this update of the NIH guidelines to account for the biosafety of synthetic nucleic acids with the understanding of the goal to reduce potential risks to laboratory workers, the public and the environment. Topigen supports the concept that synthetic oligonucleotides that are not capable of replication should be exempt under NIH Guidelines not only for basic laboratory and preclinical research, but also for human trials. Short synthetic oligonucleotides (including chemically synthesized antisense oligonucleotides and small interfering RNA- hereafter in the document referred to as oligonucleotide therapeutics) are distinct from gene therapy, and thus do not share with gene therapy products the biosafety concerns raised in the March 4, 2009 Federal Register Notice. As oligonucleotide therapeutics exert a transient effect, cannot replicate *in vivo*, do not insert themselves into host genomes, and do not pose a risk of transmissibility, these molecules act more like conventional small molecule drugs than like gene therapy modalities, and should be differentiated from therapies that use viral- or vector-mediated transformation of cells for the administration of functional genetic material. Based on an understanding of the biologic activity of chemically synthesized oligonucleotide therapeutics, Topigen supports their exemption for basic laboratory and preclinical research as proposed in the Federal Register Notice and supports their exemption also for human trials, given that the risks are sufficiently low that RAC review may hinder research and development, with little to be gained by the increased oversight.

Summary of the Proposed Actions Under the NIH Guidelines

It is proposed that oligonucleotide therapeutics, whether associated with delivery vehicles or not, be listed as exempt in the NIH Guidelines for both basic laboratory, preclinical and clinical research because they pose a low risk to investigators, the public and the environment. In response to the RAC request for written comments on specific questions for the proposed amendments to Section III-F-1 outlining exempt experiments, the following detailed remarks are offered to address concerns raised in the Federal Register Notice. The stated questions in Section III-F-1 are:

1) *Is there a sufficient distinction between the risks of basic and preclinical research with replicating vs non-replicating synthetic molecules to warrant the exemption?*

2) *Since the increased risk associated with human gene transfer is in part related to the administration of higher doses should the exemption be limited to experiments involving the handling of low quantities or doses of nucleic acids? What quantity would not be expected to pose a biosafety risk?*

3) *Are there examples of non-replicating, synthetic nucleic acid research that should not be exempt due to greater potential risks (e.g. expression cassettes for oncogenes or toxins)?*

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

Comments to the Specific Questions:

Q1. Is there a sufficient distinction between the risks of basic and preclinical research with replicating vs non-replicating synthetic molecules to warrant the exemption?

Topigen supports the view that there is sufficient distinction between the risks with replicating vs non-replicating synthetic molecules to warrant the exemption. In accordance with the NIH Guidelines there is no difference in distinguishing the *method* of creating sequences of nucleic acids in determining virulence, transmissibility and pathogenicity, which are key biosafety considerations. As such, the biosafety and health risks for synthetic, non-replicating nucleic acids are not fundamentally different from non-replicating recombinant vectors. Key to the biosafety of the oligonucleotide therapeutics however is the fact that they cannot replicate *in vivo*, nor do they pose a risk of persistence within and outside of the laboratory which could lead to transmissibility.

Topigen supports the exemption for oligonucleotide therapeutics as they are not promoter- or vector-driven with the potential to express a transgene. The oligonucleotide drug itself is not a functional gene, nor possesses promoter sequences that would enable them to be transcribed and translated. Oligonucleotide therapies do not act directly to introduce genes or change the genome itself. There is no mechanism for these drugs to self-propagate like some vector-driven therapies. In vector-driven gene therapy, integration and amplification of a functional gene permanently affects changes in target cells and potentially could even lead to changes in daughter cells. In contrast, oligonucleotide therapeutics are more like traditional drugs that are active as a result of exogenous administration, with the magnitude of effect determined by the administered dose and the concentration in the target organ. When the oligonucleotide drug is metabolized (by nucleases) and eliminated, the drug concentration decreases, and the pharmacologic effect is diminished and ultimately reversed, as is the case for small molecules. Therefore, oligonucleotide therapeutics should remain distinguished from therapies that use viral- or vector-mediated transformation of cells by the administration of functional genetic material.

Furthermore, there has not been any evidence of integration of antisense oligonucleotide drugs into cellular DNA. In *in vitro* assays, vast numbers of cells are exposed to antisense drugs at high concentrations (Molar), and the cells are allowed to replicate multiple times over the course of these experiments, allowing amplification of any potential integration event. Despite these incubation conditions in *in vitro* assays that overwhelmingly favor integration events, these assays have been uniformly negative in both bacteria and mammalian cell lines, including human cell lines. Recently compiled data from across the industry indicated that dozens of phosphorothioate or phosphodiester antisense oligonucleotide drugs have been negative in bacterial and mammalian genotoxicity assays. Because of the uniform negativity in these assays, the European Medicine Agency no longer requires *in vitro* genetic toxicity testing of some antisense drugs[1]. Given these characteristics, the potential concerns of synthesis of new forms of life or even mutant forms of existing agents with enhanced pathogenicity do not apply to oligonucleotide therapeutics.

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[1] CHMP SWP Reflection Paper on the Assessment of the Genotoxic Potential of Antisense Oligonucleotides. European Medicines Agency Pre-authorisation Evaluation of Medicines for Human Use, London, 20 January 2005 (Doc. Ref.EMEA/CHMP/SWP/199726/2004)

Q2. Since the increased risk associated with human gene transfer is in part related to the administration of higher doses should the exemption be limited to experiments involving the handling of low quantities or doses of nucleic acids? What quantity would not be expected to pose a biosafety risk?

Topigen supports the concept that the exemption of oligonucleotide therapeutics for both basic and human studies should be based not on dose of nucleic acid, but rather on the inability for replication or transmission. For oligonucleotide therapeutics, the transient duration of action without the potential for persistence due to insertion in the genome or heritability, and the inability to express a transgene should be considered as factors for their inclusion in the exemption rather than relying on dosage amounts. As previously described, oligonucleotide therapies are similar to traditional drugs with the magnitude of effect determined by administered dose and concentration in target organs. There are approved and investigational drugs that are currently not regarded as posing a biosafety risk although they too function by alterating endogenous protein expression by various means, including effects on DNA replication, RNA transcription and translation. Thus, rather than the dose of the nucleic acids being tested, the ability of the nucleic acid to replicate *in vivo* should be used as a criterion for RAC review. Also handling of large quantities of oligonucleotides is currently undertaken with the same safety and protection measures for small molecules.

Q3. Are there examples of non-replicating, synthetic nucleic acid research that should not be exempt due to greater potential risks (e.g. expression cassettes for oncogenes or toxins?)

Oligonucleotide therapeutics can alter protein expression patterns by binding to a specific nucleic acids sequence to alter DNA replication, transcription of DNA in to RNA, or translation of RNA into protein. Once the agent is removed the effect is reversed. Concerns have been voiced that modifications which improve penetration of cell membranes by synthetic nucleic acids so as to improve delivery may also lead to increased risk (e.g. topical agents, liposomal formulations or lipid complexes). Topigen would argue that modifications that improve delivery of oligonucleotide therapies into cells do not increase the low biosafety risk of the therapies based on the fundamental properties outlined in the preceding sections; the improved delivery does not affect the inability of the oligonucleotides to replicate, the inability for transmission from generation to generation, and the persistence of oligonucleotide. Thus, Topigen supports exemption of oligonucleotide therapeutics with or without the use of delivery vehicles.

Topigen does support RAC review for any synthetic or naturally occurring nucleic acids that are promoter- or vector-driven with the potential to express a transgene and or to replicate in the host cells.

Q4. For human gene transfer research, are there classes of non-replicating molecules that should be exempt due to lower potential risks (e.g. antisense, RNA, RNAi etc?). If so, what criteria should be applied to determine such classes?

Topigen does support the exemption of classes of non-replicating molecules due to lower potential risks, and would argue that these oligonucleotide therapies not be classified as "gene therapy". In general, the target for antisense and siRNA drugs is RNA, not the genomic DNA. These drugs induce destruction of target mRNA via enzyme-mediated mechanisms involving RNAse H (most antisense) or RISC (siRNA). These mechanisms are dependent on a natural process in which the RNAse H or RISC enzymes destroy any RNA (in this case target mRNA) that is hybridized to the oligonucleotide drug. These enzymes are incapable of cleaving DNA, so it is not possible to affect the genome through an RNAse H- or RISC-based mechanism.

It is possible that antisense and siRNA drugs interact with DNA, but the effects are transient. Some antisense drugs can nestle into the DNA helix and form triplex structures with DNA. This process has been thoroughly evaluated as a potential therapeutic modality, but like other antisense activity, the interaction is transient and does not affect the fidelity of transcription, nor does it induce changes in the genomic sequence. It simply blocks transcription and does not induce heritable changes. In theory, in triplex formation, the antisense drug binds to its cognate sequence in genomic DNA and sterically blocks transcription. This type of interaction is not favored thermodynamically for a number of reasons, and it is unclear if it is possible at physiologic pH and temperature. Like all other forms of exogenously administered antisense drugs, the effects would be transient and reverse as the antisense drug is metabolized and cleared. Again, like the other mechanisms of antisense activity, these interactions should not be the basis for a RAC review requirement as gene therapy because they do not alter the genome.

Another concern raised in the Proposed Action under the NIH Guidelines in the context of oligonucleotide therapies to potentially induce an immunological response. This concern is not unique to oligonucleotide therapeutics, being shared with small molecules drugs, biologics and vaccines that do not pose biosafety risks. Thus, the potential to induce an immune response should not prevent classification of oligonucleotide therapeutics as exempt under the NIH Guidelines for human studies.

In summary, oligonucleotide therapeutics do not contain sufficient genetic information to be functional genes, nor do they insert into the genome to produce heritable genetic changes or damage. Their effect is also transient and reversible just like small molecules therapeutics. Therefore, Topigen proposes that oligonucleotide therapeutics be classified as exempt under NIH Guidelines for human clinical trials.

Final Comments

To require RAC review of human trials using oligonucleotide therapeutics would run the risk of impeding development of promising drugs for medical conditions that may be otherwise untreatable with other medicinal products. Given the nature of oligonucleotide therapeutics, there is little scientific justification for addition of RAC oversight to the existing adequate regulatory supervision. Topigen supports exemption under NIH Guidelines of oligonucleotide therapeutics for basic laboratory, preclinical research and human clinical trials.

We appreciate the opportunity to share our comments with respect to the Proposed Actions Under the NIH Guidelines.

Sincerely,

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Director, Preclinical Development

Topigen Pharmaceuticals

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Canada

Dr. Rosanne Seguin

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Michael Malamy
Professor

Tufts University School of Medicine

The opinions expressed in this memo are those of the author and are not necessarily the official position of the Tufts University School of Medicine.

From: Michael Malamy [mailto:michael.malamy@tufts.edu]
Sent: Wednesday, April 22, 2009 2:47 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Proposed changes to guidelines

To whom it may concern:

I am writing to express my objections to the proposed changes in the NIH Guidelines for Recombinant DNA, especially section III-A-1. The use of antibiotic resistance markers is central to my NIH supported work on two intestinal organisms, Escherichia coli, and Bacteroides fragilis. We, and others, have characterized the existence of intra-species transfer of plasmids and transposons between natural isolates of these strains. On this basis, our experiments have been exempt from the guidelines. We also use shuttle vectors that contain antibiotic resistance markers that are active in one strain or the other. All of our current genetic analysis depends on the use of these shuttle-vectors, and transmissable suicide vectors that deliver DNA from one species to the other.

It is also agreed, that these organisms are natural DNA exchangers with a multitude of bacterial species in the intestinal environment. Indeed, genes for antibiotic resistance in B.fragilis, a Gram-negative bacillus, have, more often than not, been shown to have a closely related gene in the Gram-positive cocci, a very distant relative ; thus these genes are being exchanged "in the wild" with great frequency.

It is for these reasons, that I believe it is not necessary to include "natural exchangers" and their drug resistance genes on the list of organisms that warrant surveillance by the NIH guidelines. We are already bound by a restriction against the use antibiotics for which resistance mechanisms have not been discovered in the organisms that we use. That should afford adequate control, if this is thought to be a problem.

It would be unwise to implement the proposed revisions in section III-A-1, the revisions are not scientifically justified and it will only clog the existing system with unnecessary tasks.

Respectfully,

Michael Malamy
Professor
Tufts University School of Medicine

Abraham L. Sonenshein
Professor and Acting Chair
Department of Molecular Biology and Microbiology

Tufts University School of Medicine

From: Linc Sonenshein [mailto:linc.sonenshein@tufts.edu]
Sent: Friday, April 10, 2009 9:22 AM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Proposed Revision of NIH Recombinant DNA Guidelines

I am writing to register my opposition to the proposed change in section III-A-1 of the Recombinant DNA Guidelines. The proposed change is unnecessary, has no justification based on any demonstrated hazard, and would bring to a halt major aspects of the study of microbial pathogenesis. The change would also be very difficult for local IRBs to implement and would flood the NIH Director's office would applications that would take eons to review.

If there is any scientific evidence to support the necessity for the proposed change, it should be made available to the community for evaluation.

Sincerely yours,

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Joshua Fierer, M.D.
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From: Joshua Fierer [jfierer@ucsd.edu]
Sent: Thursday, April 09, 2009 4:10 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Federal Register / Vol. 74, No. 41 / Wednesday, March 4, 2009 / Notices

I wish to object to the change in the rules concerning the transfer of antibiotic resistance genes into bacteria that is proposed in the paragraph below. This methodology is so widespread and so useful in studies of microbial pathogenesis that either the NIH intends to seriously cripple pathogenesis research or to seriously expand the bureaucracy in order to expeditiously review the thousands of that will have to be reviewed each year. Furthermore, I can imagine the paper work burden that will be imposed on investigators to keep track of each approval as presumably an approval will be needed for every transposon that is used to create a mutation or a selectable marker. Since there is no evidence that people or animals have been harmed by current practices, what justification is there for such a far-reaching change in the rules?

I should also point out that these methods are used to teach medical students about mechanisms of acquired antibiotic resistance, at least in my School of Medicine. Every year we have a Microbiology Laboratory exercise in which students transfer an antibiotic resistance plasmid from a E. coli to a Salmonella. The students love the "experiment" and it is a graphic illustration of clinically and epidemiologically important principle of microbiology and infectious diseases. As I read the proposed change we would have to get NIH approval to do this student experiment.

Finally, what is intended by the phrase "not the drug of choice"? Does that mean that transfer of any drug resistance genes would require pre-approval? For instance, aminoglycosides should never be used to treat Salmonella infections, so would kanamycin resistance be reviewed? What about tetracyclines? Who will decide where to draw the line?

For all these reasons I am not in favor of the proposed changes.

Sincerely,

Joshua Fierer, M.D.
Michael and Marcie Oxman Professor of Infectious Diseases
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Departments of Medicine and Pathology
Chief of Infectious Diseases
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Section III–A–1 will clarify that all experiments involving the transfer of a drug resistance trait to a microorganism will be subject to RAC review and NIH Director approval if the microorganism's acquisition of the trait could compromise public health. The changes will clarify that the microorganism's ability to acquire the trait naturally is not relevant to the safety of the experiment, that the provisions apply even if the drug at issue is not considered the ''drug of choice,'' and that adverse effects on population subgroups need to be considered.

Anthony T. Maurelli, Ph.D
Professor

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May 4, 2009

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National Institutes of Health
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This letter is in response to the proposed revisions published in the Federal Register, Volume 74, Number 41 regarding Section III-A-1 of the NIH Guidelines for Research Involving Recombinant DNA Molecules. Specifically, I am opposed to the proposed revisions to ignore both the microorganism's natural ability to acquire antibiotic resistance and whether the drug in question is the drug of choice for treatment when considering the safety of an experiment. I believe that the proposed revisions are ill-conceived, needlessly restrictive and can potentially have a broad and detrimental effect on the study of pathogenic microorganisms using recombinant DNA techniques.

First, the proposed revisions are not supported by any scientific data that the current regulations pose a safety hazard to investigators or the public. The published notice states only that "the current language has raised concerns" about how to identify experiments that require heightened review because of the transfer of drug resistance traits to an organism. It does not specify what those concerns are. Consequently there is no way of assessing whether the proposed revisions will address those concerns. If the concerns are confusion about what experiments need to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC), perhaps they can be assuaged by better defining the conditions of natural acquisition of drug resistance by the microorganism in question. However, what the proposed revisions appear to do is to resolve the issue by essentially making all microorganisms subject to the heightened review.

Second, the wisdom underlying the crafting of the original guidelines in the 1970s was to be flexible and reduce restrictions on experiments that introduced foreign DNA into a microorganism as more information became available about the consequences of such experiments. Thus, many experiments that were initially prohibited because of perceived and speculated risks became acceptable as we learned more about the actual risks. A similar science-based approach should also be applied to any modification of the guidelines that would further restrict experiments using recombinant DNA. The proposed revisions place the NIH in the awkward position of potentially prohibiting the laboratory transfer of drug resistance to microorganisms while the very same transfer events are occurring freely in nature.

Third, the wording of the proposed revisions is so vague that strict interpretation of the new provisions would effectively subject many experiments to RAC review and NIH Director

approval: "all experiments involving the transfer of a drug resistance trait to a microorganism will be subject to RAC review and NIH Director approval if the microorganism's acquisition of the trait could compromise public health." What constitutes a threat to public health? Who makes that determination? What evidence needs to be presented to support any such a claim? One can imagine that the proposed revision would require even an experiment that introduces a plasmid cloning vector encoding ampicillin resistance into *Escherichia coli* K-12 to be reviewed. Do we know that the acquisition of ampicillin resistance by *E. coli* K-12 will not compromise public health? Probably not, but what evidence does an investigator need to provide to support this contention? Would the acquisition of tetracycline resistance by *Shigella flexneri* compromise public health? Absolutely, since this microorganism is a frank pathogen. In fact, the acquisition of resistance to tetracycline, sulphonamides, trimethoprim-sulfamethoxazole, streptomycin, amoxicillin, amoxicillin-clavulanic acid, and chloramphenicol by strains of *Shigella* has already compromised public health. However, none of these drug resistances were acquired by *Shigella* due to laboratory manipulation of the organism. Natural transfer of multiple drug resistance to *Shigella* was first reported in Japan in the 1950s. In the subsequent decades, natural transfer of drug resistance was described in a wide range of pathogenic bacteria. The evidence is clear: bacteria exchange DNA in nature and antibiotic resistance genes are widespread in the bacterial kingdom. There is no benefit to public health to subject to review (and possibly prohibit) an experiment that involves laboratory transfer of a plasmid encoding a drug resistance gene to a bacterium that is perfectly capable of acquiring the same drug resistance gene on its own outside the laboratory. An investigator working on the pathogenic 2457T strain of *Shigella flexneri* 2a (which is sensitive to all of the above antibiotics) would be placed in the absurd position of requesting permission to introduce a plasmid encoding resistance to any of these drugs into 2457T when *Shigella* strains causing dysentery around the world have already acquired and continue to share these drug resistances by horizontal gene transfer in nature. Any Institutional Biosafety Committee (IBC) reviewing such an experiment would be justified under the new revision to refer this experiment to the RAC because this strain of *Shigella* does not have any genes for drug resistance (and thus may pose a threat to public health) even though the same microorganism in nature would very quickly acquire such resistance genes.

Fourth, the proposed revisions would potentially subject some of the most powerful genetic tools for understanding bacterial pathogenesis to RAC review and NIH Director approval. Techniques such as transposon mutagenesis, signature-tagged mutagenesis, and *in vivo* expression technology are, by design, genetic screens that introduce antibiotic resistance markers into drug sensitive pathogens in order to identify genes that are essential for virulence, survival and/or colonization in an animal host. Under the proposed revisions, these experiments may be prohibited even though isolates of the same pathogen expressing the same drug resistance markers exist in the clinical setting due to natural horizontal gene transfer.

Fifth, the deletion of consideration of whether the drug resistance gene in question is effective against the "drug of choice" for the microorganism being studied will further restrict important research. Conceivably any gene that encodes resistance to any drug which is effective against any pathogen, whether this drug is used in clinical practice or not, would be subject to the proposed rules and referred to the RAC. An IBC would presumably be justified in recommending such action if even a single report of even one use of the drug to treat infection with the microorganism were reported in the literature. The proposed revision leaves no room for the reasoned consideration of clinical data relative to real world treatment of infections with the

microorganism, i.e. the matter of the "drug of choice" is no longer considered relevant under the proposed revision.

In summary, the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules are not justified. The proposed revisions are not supported by any scientific evidence that demonstrates a need for the proposed revisions to protect the public health. In the absence of any data that substantiate the need for the proposed revisions, the potential adverse consequences of implementing these revisions provides another compelling argument against the proposed revisions.

Thank you for your consideration of these comments.

Sincerely,

Anthony T. Maurelli, Ph.D.
Professor

Leland S. Pierson, Ph.D
Chair, Institutional Biosafety Committee
Professor, Plant Pathology

The University of Arizona

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Arizona.

THE UNIVERSITY OF ARIZONA.

Research Compliance and Policy

Office of the Vice President for Research, Graduate Studies and Economic Development

Institutional Biosafety Committee

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April 30, 2009

Office of Biotechnology Activities
National Institutes of Health
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Dear Sirs:

As Chair of the University of Arizona's Institutional Biosafety Committee (IBC), I am enclosing comments that we have concerning the proposed actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. This was requested and published in the Federal Register, Vol. 74, No. 41, Wednesday, March 4, 2009. Specifically we object to the proposed changes in Section III-A-1, which unnecessarily change the extent of review for experiments involving drug resistance transfer. We hope that the NIH will agree that these alterations are more likely to hinder rather than serve the public benefit.

Sincerely,

Leland S. Pierson III

Leland S. Pierson, Ph.D.
Chair, Institutional Biosafety Committee
Professor, Plant Pathology

COMMENTS FROM THE INSTITUTINAL BIOSAFETY COMMITTEE (IBC) OF THE UNIVERSITY OF ARIZONA, TUCSON, ARIZONA, ON THE PROPOSED ACTIONS UNDER THE NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES AS PUBLISHED IN THE FEDERAL REGISTER, VOL. 74, No. 41

Sections I-B and III-E-1

We have no problem with the proposed revisions to Section I-B as they appear to clarify the means by which NIH Guidelines are applied to research with synthetic nucleic acids. Likewise, the proposed changes to Section III-E-1 seem reasonable.

Section III-A-1

We object to the proposed changes in Section III-A-1, which unnecessarily change the extent of review for experiments involving the transfer of drug resistance elements to microorganisms. Selectable resistance markers are routinely used around the world to inactivate genes in pathogenic bacteria in order to determine the role of the gene in pathogenesis. To fulfill molecular Koch's postulates, the mutation must be verified by introduction of a non-mutated copy of the gene on a plasmid and the demonstration that this restores the original phenotype. The presence of the plasmid in the cell is maintained by antimicrobial selection. As this is the foundation of most molecular genetic analyses, the study of bacterial genetics without the use of antimicrobial selection is unimaginable.

The current guidelines do not require RAC review if the microorganism can acquire the trait naturally. This has worked extremely well in the past and there is no reason to believe it will not continue to work well in the future. The proposed deletion of the phrase "that are not known to acquire the trait naturally" will require all research of this type be subject to RAC review and NIH Director approval. This would be a devastating blow to microbiological research and public health. It is very unlikely that any REAL risk is posed by use of antimicrobial resistance markers in the study of bacteria and bacterial diseases, in that there exists absolutely no documentation of adverse outcomes despite hundreds of thousands of experiments and decades of use. Scientists in both the public and private sectors, who focus on the emergence of multiple-resistant bacteria, believe this is due to indiscriminant use of antimicrobials by the healthcare industry. Thus, strains constructed in the course of basic research seem unlikely to be a threat.

The supposed purpose of the revision is to clarify the current guidelines used by IBCs, but we think it will have the opposite effect. Broadening the range of concern to include consideration of possible rare uses of an antibiotic that is not a "drug of choice" will be confounding at best. Literal interpretation of the changed regulation will lead to major and unnecessary delays in project approval and bring some legitimate and harmless, to say nothing of beneficial, work to a halt.

In view of the importance of antimicrobials in bacteriology research and the effectiveness of existing means for protection of people from engineered antimicrobial-resistant strains, this revision of the RAC Guidelines is misguided and will hinder rather than serve the public interest.

Alan Barbour, M.D.
Director, Pacific-Southwestern Regional Center of Excellence for Biodefense & Emerging Infectious Diseases

University of California, Irvine

From: Alan Barbour [abarbour@uci.edu]
Sent: Thursday, April 09, 2009 4:24 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Comment on proposed revisions of NIH guidelines for recombinant DNA
Office of Biotechnology Activities
National Institutes of Health, PHS, DHHS

Re: Federal Register Vol. 74; March 4, 2009; pages 9411-9421

I write in specific support of the following change in the guidelines with respect to the deliberate transfer of a drug resistance trait to microorganisms, in particular the added language about certain groups and subgroups.

'Section III–A–1-a is proposed to be amended to: ‘‘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 (see Section V–B, Footnotes and References of Sections I–IV). 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.’’ '

An example of an experiment that was permitted without RAC review but which would reasonably fall under the new guidelines was the deliberate introduction of erythromycin resistance trait into the Lyme disease agent, Borrelia burgdorferi (Sartakova M et al. Development of an extrachromosomal cloning vector system for use in Borrelia burgdorferi. Proc Natl Acad Sci U S A. 97:4850-5, 2000). Before these experiments were carried out, erythromycin resistance was not documented to occur naturally. Erythromycin and other macrolide antibiotics are not the first choice for therapy of Lyme disease, but they can be effective and are considered alternatives to tetracyclines and certain beta-lactam antibiotics. They would certainly rise in preference over tetracyclines in the case of a pregnant or nursing woman or child of less than 8, and over beta-lactam antibiotics in the case of penicillin or cephalosporin allergy. Erythromycin is also generic and affordable in countries where the cost of alternative antibiotics still on patent would be prohibitive. There was already a suitable resistance trait available for transfer into B. burgdorferi: kanamycin and other aminoglycosides, which are not recommended under any circumstances for treatment of Lyme disease.

As director of a Regional Center of Excellence for Biodefense and Emerging Infections, I am also aware of a number of other pathogenic bacteria, the treatment of which could be compromised for certain groups, including individuals in some other countries, if some drug resistance traits were introduced. In many cases these experiments are justified in spite of the risk, but this should be assessed at a higher level by an advisory group with greater expertise and experience than is typical for most IBC's.

Alan Barbour

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The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of California at Davis.

Office of Biotechnology Activities
National Institutes of Health

To whom it may concern,

I fully agree with ASM's assessment that the "proposed changes in Section III-A-1 would have a chilling impact on microbiological research where antibiotic resistance is routinely used in molecular and genetic studies".

In my field of study, Salmonella, the emergence of multiple drug resistance results from the use of antibiotics in humans and in agriculture, which occurs globally. In contrast, antibiotic resistance introduced for purposes of basic research in the laboratory or in laboratory animals is not a source of drug resistance in clinical isolates. Antibiotic resistance markers are essential tools for microbiology research, which provides important benefits, but no measurable risks for increasing the prevalence of naturally occurring antibiotic resistant Salmonella clinical isolates.

I strongly urge the Committee considering NOT to delete the phrase ³that are not known to acquire the trait naturally,² in Section III-A-1. Deleting this sentence would do nothing to improve the incidence of antibiotic resistance in clinical isolates. However, it would impede research that is ultimately needed to address this problem by developing alternatives to antibiotics for treatment.

Sincerely yours

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Michael Glotzer
Department of Molecular Genetics and Cell Biology

University of Chicago

From: Michael Glotzer [mailto:mglotzer@uchicago.edu]
Sent: Thursday, April 23, 2009 4:47 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Revision to Section III-A-1 Major Actions Under the NIH Guidelines

I am in full support of the ASM statement (http://www.asm.org/Policy/index.asp?bid=64123) concerning this revision.

Strict interpretation of this policy will greatly impede biological research and ultimately impair public health.

Best Regards,

Michael

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Alison Weiss
Professor; Molecular Genetics, Biochemistry and Microbiology

University of Cincinnati

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Cincinnati.

Well intentioned legislation can have severe, negative consequences.

No where is this more apparent than the restrictions imposed on biomedical researchers following the anthrax episode. I am studying Shiga toxin produced by *E. coli* O157:H7. Children die from this disease each year, and there is a great need to develop cures. However research in this area has been significantly hampered due to recently enacted limitations on recombinant DNA research.

Current restrictions on pathogenic microbes and new proposed restrictions regarding antibiotic resistance genes should be re-evaluated because:

1. All organisms from scientific research are destroyed after each experiment. Legitimate research poses no danger to the public.
2. Restrictions on legitimate scientific research slow progress toward the development of cures, creating unnecessary suffering.
3. Pathogens are everywhere in the environment (for example *E. coli* O157:H7 is likely to be in the food you buy at your local grocery store). Locking-down research labs does not lock up the pathogenic organisms.
4. Foreign researchers are making rapid progress while US scientists get fingerprinted and FBI checked. It is easy to envision a scenario where the US becomes entirely dependent on the generosity of foreign nations for new vaccines because our scientists were unable access important strains in a timely manner.

There is no need to enact more restrictive regulations on legitimate scientific research. I suggest that oversight of Recombinant DNA should be returned to the local oversight committees.

In addition, current regulations need to be revisited to balance public safety with the public's right to the cures from biomedical research.

Sincerely,

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Dr. Julie Zilles

University of Illinois

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Illinois.

-----Original Message-----
From: Julie Zilles [mailto:jzilles@illinois.edu]
Sent: Thursday, April 23, 2009 2:33 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: Julie Zilles
Subject: comment on proposed changes to NIH RDNA guidelines

I would like to register an objection to the proposed change to Section III-A-1, regarding the deliberate transfer of drug resistance to a microorganism. The potential public health risk associated with this transfer is rare and largely concentrated to cases involving particular pathogenic microorganism/drug combinations. Rather than clarifying the identification of those cases, the current language serves to broaden the research encompassed by this regulation.

These broad restrictions on a fundamental molecular tool have a substantial potential impact on scientific progress and on the medical and environmental benefits associated with that progress and present in my opinion a much more substantial threat to public health than the experiments they are designed to prevent.

Thank you for your consideration.

Dr. Julie Zilles

Michael Vodkin
Biological Safety Professional
Biological Safety Section

University of Illinois at Urbana Champaign

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Illinois at Urbana Champaign

Dear OBA Director,

I am submitting the following comments in response to the March 4, 2009 Federal Register proposed revisions to the *NIH Guidelines for Research involving Recombinant DNA*.

1. I mainly agree with the proposal to change Section IB to accommodate the applicability and versatility of synthetic nucleic acids to basic and pre-clinical research. (UIUC does not have a clinical program, so no comments will be made for this area.) A clear and appropriate statement has been made for replicating synthetic nucleic acids, but there is no clear guidance provided for non-replicating synthetic nucleic acids that can pose personnel risks. Examples of this class include non-specific effects with poly I:C and targeted effects of specific siRNAs that at sufficiently high concentration could adversely affect physiology or metabolic processes. An educational effort (a few sentences in the Guidelines), rather than registration, would be more appropriate to advise researchers of the risk of auto-inoculation .

2. I am neutral with respect to the revisions to require more stringent oversight in Section III-A-1 when introducing drug resistance into microorganisms, if such introduction would compromise treatment of infected people or animals. The two proposed revisions are to extend oversight to drug resistances that are known to be naturally acquired by the host microorganism and to take into consideration whether a subgroup of the population would be jeopardized by the recombinant drug resistance gene.

   Strict compliance with either the original or revised version of III-A-1 will lead to considerable delay to IBC-approved registrations and overload NIH/OBA, even with the proposed administrative triage policy. Antibiotic resistance markers are routinely used to select for recombinant microorganisms. Most of these commercially available selection markers involve drugs that would not be the main-line or alternative treatment choice. An alternative approach is to limit III-A-1 to recombinant DNA hosts that are classified as Risk Group 3, Risk Group 4, or any Select Agent that is Risk Group 2.

3. I agree with the revised Section III-E-1 to require BL-1 containment for high risk viral nucleic acids in tissue culture that contain less than ½ of the same family genome and that are replication-incompetent. Would you clarify the containment level that will apply for Risk Group 2 viruses in tissue culture with ½ to 2/3 of the same family genome being present.

There seems to be a typographical error on the right-hand column of page 9415. A “not” was omitted in the second part of “…with synthetic NA that can not replicate or that were derived from molecules that can replicate.”

Michael Vodkin

Biological Safety Professional
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Peter R. Williamson, M.D., Ph.D
Associate Professor of Medicine, Pathology, Microbiology and Immunology

University of Illinois at Chicago School of Medicine

From: Peter Williamson [mailto:prw@uic.edu]
Sent: Wednesday, April 22, 2009 2:25 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Section 1-B comments

- Pertaining to: Section 1-B- Broadening the scope of the NIH Guidelines, which currently cover laboratory and clinical research involving DNA molecules created via recombinant techniques. NIH proposes to encompass nucleic acids that are synthesized chemically or by other means without the use of recombinant technology.

Response. We currently use thousands of olignucleotide primers in our research on a daily basis. They have absolutely no danger to anyone. Adding more unfunded mandates such as the one proposed would tremendously reduce our capacity to do research. Keeping track of oligos and reporting would cause to our laboratory an estimated increase in expenditures of $10,000 per year and/or reduce our research efforts by about 5%. Applying this to the NIH budget, we estimate that this will reduce funding by an extimated $5 billion dollars and markedly reduce the efficiency of the research infrastructure. This will significantly reduce the bottom line research efforts and result in loss of life by patients critically dependent on our research. You have provided no justification for the lives that will be lost from deferred research due to this unnecessary regulation.

Peter R. Williamson, MD/PhD
Associate Professor of Medicine, Pathology, Microbiology and Immunology
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Dr. Robert D. Perry
Professor of Microbiology
Department of Microbiology, Immunology and Molecular Genetics

University of Kentucky

From: Perry, Robert [rperry@email.uky.edu]
Sent: Friday, April 10, 2009 8:48 AM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Proposed Revision to NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)
Office of Biotechnology Activities:

I am writing to indicate I fully support the position of the American Society for Microbiology (ASM) on the proposed changes involving recombinant DNA molecules and antibiotic resistance transfer. I have copied that response below in the hope that repetition will make more of an impact.

All too often in the past decade or so "biosafety regulations" have been made or changed without a very careful consideration of 1) real life history and experience; and 2) the negative impact on responsible biological research.

I repeat the ASM statement question - "Given that no documented harm has come from laboratory research using antibiotic resistance markers, the question must be asked as to why a change in the Guidelines is warranted." It would be wonderful is Biosafety regulation changes would follow the rule – "If it's not broken don't fix it."

Removing a clear guideline will inevitably lead to a wide range of interpretations by individual IBCs. So research at some institutions may see very little affect while others will see rulings that make recombinant DNA research almost impossible. This range of rulings will not improve Biosafety and will have an extremely negative affect on research.

As a relevant example, an official at my institution decided several years ago that DNA primers (12-30 base pairs of DNA) sent or received from molecular biology companies for sequencing and other uses needed to be classified as hazardous materials. When the protests from researchers started, this official claimed that he could rule that wool was a hazardous material according to his interpretation of regulations. Fortunately, our IBC and others forced this decision to be revoked. Yet, there was a period of several weeks when a local ruling made it difficult to conduct routine research. By making the guidelines more ambiguous and increasing the types of activities that need approval you are inviting similar abuses nationwide.

I hope you will give the ASM position (and I am sure the position of nearly all responsible researchers) serious consideration and keep the current NIH guidelines on antibiotic resistance transfer in microorganisms that has worked fine for decades.

Sincerely,

Dr. Robert D. Perry

Professor of Microbiology
Department of Microbiology, Immunology, and Molecular Genetics
University of Kentucky

April 9, 2009 - ASM Comments on Revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
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Bethesda, MD 20892-7985

Subject: Response to request for comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register*, 9411-21

The American Society for Microbiology (ASM) is submitting the following comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register*, 9411-21:

Revision to Section III-A-1 Major Actions Under the NIH Guidelines. The proposed revised section states that all experiments involving "the deliberate transfer of a drug resistance trait to a microorganism, if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine or agriculture," will receive RAC review and NIH Director approval. The current NIH Guidelines state that if the microorganism is known to acquire the trait naturally, then transfer of the drug resistance may not need RAC review. The NIH is now proposing to delete the phrase "that are not known to acquire the trait naturally," in Section III-A-1.

Further discussion of this stringent review policy and assessment of the presumed risk to the public and the environment posed by antibiotic resistance markers in basic and pathogenic bacteriology research is needed. If interpreted literally, as it likely will be, this language could have a chilling impact on microbiological research where antibiotic resistance is routinely used in molecular and genetic studies. Given that no documented harm has come from laboratory research using antibiotic resistance markers, the question must be asked as to why a change in the Guidelines is warranted. Any change in Section III-A-1 should clarify and narrowly focus on areas of concern. The proposed language does the opposite. By broadening the activities that require approval, it will have an adverse impact on microbiological research and public health.

The stated purpose of the revision to Section III−A−1 is to clarify the current guidelines for local Institutional Biosafety Committees (IBCs). The NIH Guidelines are based on the

premise that local oversight is the best approach to biosafety. However, the work of the IBCs will be more complicated if the proposed change is adopted. While we agree that whether or not an organism acquires the trait naturally is not the critical factor in evaluating the safety of the experiment, broadening the range of concern to include consideration of possible rare uses of an antibiotic that is not a "drug of choice," will only confound the work of the IBCs.

Selectable antibiotic resistance markers introduced into bacteria via plasmids, transposons, or by homologous recombination are the most conventional, versatile, and widely used tools in the study of bacterial pathogenesis and bacterial physiology. Such antibiotic resistance genes have been used to replace or inactivate bacterial genes to elucidate key physiological or pathogenic traits. Such mutant traits are then complemented with a replacement copy of the gene borne on a plasmid that is maintained under antibiotic selection, an essential step in fulfillment of Molecular Koch's Postulates. The applications for antibiotic resistance selection are so numerous that it is impossible to envision the study of bacterial genetics without the use of antibiotic selection markers. The real question is whether such antibiotic resistance markers pose an actual risk in treatment of infections with the bacterial strains and pathogens we study.

Previously, researchers took into consideration the likelihood that the selection agent would be clinically used as a therapy for infection. However, concerns about multiply resistant agents of all types, the "drugs of choice" and the threat to the public posed by resistant bacteria are more difficult to determine. Nonetheless, the emergence of multiple drug resistance in bacteria is universally regarded as a product of the indiscriminant use of antibiotics in humans and in agriculture worldwide. Therefore, bacteriologic strains developed during the course of basic scientific research and tested in vitro or in vivo in laboratory animal models are relatively unlikely to pose any threat to the population or environment at large.

Given the value of antibiotics in the study of bacteria, and the mechanisms already in place through other federal regulations to protect the public from agents in the research laboratory environment, we consider this revision to the RAC Guidelines as ill-defined in purpose and counterproductive to the generation of helpful science in the interest of public health.

The ASM supports the proposed revisions to Section I-B, Basic Research with Recombinant and Synthetic Nucleic Acids, which clarifies the applicability of the NIH Guidelines to research with synthetic nucleic acids, and Section III-E-1, Experiments Involving DNA Molecules Containing No More Than One Half of the Genome of Any Eukaryotic Virus, which changes the level of review for recombinant or synthetic experiments involving more than half but less than two thirds of the genome of certain viruses.

We appreciate the opportunity to comment on the proposed changes to the RDNA Guidelines.

Sincerely,

Alison O'Brien, Ph.D., President, ASM
Ronald M. Atlas, Ph.D., o-Chair, Committee on Biodefense
Kenneth I. Berns, M.D., Ph.D., o-Chair, Committee on Biodefense

James B. Kaper, Ph.D
Professor and Chair
Department of Microbiology & Immunology

University of Maryland School of Medicine

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Maryland School of Medicine.

Sinclair, David (NIH/OD) [C]

From: Sinclair, David (NIH/OD) [C]
Sent: Friday, May 29, 2009 11:33 AM
To: Sinclair, David (NIH/OD) [C]
Subject: FW: Revision to recombinant DNA guidelines

From: James Kaper [mailto:jkaper@medicine.umaryland.edu]
Sent: Sunday, May 03, 2009 2:58 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: Revision to recombinant DNA guidelines

I am writing regarding the proposed changes in the NIH Guidelines for Recombinant DNA experiments published in the Federal Register March 4, 2009, vol. 74, no. 41. I support the proposed changes to sections 1-B and III-E-1. However, I strongly object to the proposed changes to section III-A-1 regarding the introduction of drug resistance traits into microorganisms. I question why a change in this section is necessary since I am unaware of any harm that has resulted from laboratory research using antibiotic resistance markers. Expanding the prohibited experiments to include possible rare uses of an antibiotic that is not a "drug of choice", as is proposed in these revisions, would lead to a chaotic and inconsistent applications of these guidelines among different IBCs. The study of the mechanisms by which bacteria caused disease has benefitted enormously by the use of antibiotic resistance markers in controlled laboratory settings over the past 30 years. Such applications have directly led to the development of new vaccines and therapeutic interventions. The proposed changes in the guidelines would have a dramatic inhibitory effect on the whole field of microbial pathogenesis for no apparent gain in safety. I strongly urge that the current guidelines regarding the use of antibiotic resistance genes remain unchanged or if they are changed, that explicit language be included to specify which antibiotic resistance genes are prohibited for which bacterial species. The proposed language changes are too broad and vague to be of any value but they would greatly hamper research in this area.

Sincerely,

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John C. Drach, Ph.D

University of Michigan

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Michigan.

From: jcdrach@umich.edu [mailto:jcdrach@umich.edu]
Sent: Wednesday, April 22, 2009 2:53 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: jshoemaker@asmusa.org
Subject: Proposed Action on RDNA Experiments Involving Drug ResistantTraits

Based upon a reading of the proposed amendments to the NIH Guidelines provided by the American Society for Microbiology, I'm concerned that the regulations will do more to inhibit legitimate, useful research than to detect or deter potentially harmful work. Experiments involving deliberate transfer of a drug resistance trait to a microorganism is a very useful pathway to understanding drug action, microbial biology, and can be an essential part of new drug discovery. All of these and more would be made more difficult by expanding regulations.

Those wishing to use "research involving DNA molecules created via recombinant techniques" for nefarious purposes would not come to the attention of regulatory bodies under any conditions. Thus the regulatory burden would fall on legitimate scientists who do not need additional regulation.

Or so it seems to me.

Sincerely,

John C. Drach, Ph.D.
University of Michigan

Penelope J. Padget, Ph.D.,M.P.H.
Associate Biological
Safety Officer
Environment, Health & Safety

University of North Carolina at Chapel Hill

From: Padgett, Penelope J. (Environmental Health & Safety) [mailto:pjpadgett@ehs.unc.edu]
Sent: Friday, May 01, 2009 2:13 PM
To: Office of Biotechnology Activities (NIH/OD)
Cc: Howard, Deborah (Environment Health & Safety)
Subject: Comments on proposed Guidelines changes

Please find attached comments on the proposed changes to the NIH Guidelines for Research Involving Recombinant DNA Molecules. My contact information is below if there are any questions concerning these comments.

PJ Padgett

Penelope J. Padgett, Ph.D., M.P.H.
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Dear Penny,

This is the section that concerns our research directly:

Section III–E–1 is proposed to be amended to state: ‘‘Recombinant and synthetic nucleic acid molecules containing no more than half of the genome of any one Risk Group 3 or 4 eukaryotic virus (all viruses from a single Family being considered identical [*see* Section V–J, *Footnotes and References of Sections I–IV]*) may be propagated and maintained in cells in tissue culture using BL1 containment (as defined in Appendix G) provided there is evidence that the resulting nucleic acid in these cells is not capable of producing a replication competent nucleic acid. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III–D–3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA viruses or Defective Animal or Plant DNA or RNA viruses in the Presence of Helper Virus in Tissue Culture Systems* should be used. The nucleic acids may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than one-half of a genome.’’

Here are my comments on the proposed changes:

Research on defective replicon particles (VRP) derived from Venezuelan equine encephalitis virus (VEE) has been ongoing under NIH funding for the past 15 years. The defective genomes contained in the VRP include less than 2/3 but more than 1/2 of the virus genome, including primarily sequences that encode the virus replicase and that are absolutely required for VRP function. Many sensitive tests of our preparations of VRP have shown that they are unable to produce viable progeny virus, and are incapable of spreading to a neighboring cell. VRP-based vaccines have been shown to be safe in multiple Phase I human trials. The critical function of VRP rests on the ability to self-amplify their deleted RNA genomes and express an inserted non-VEE gene for the induction of an immune response. To do this requires nearly 2/3 of the VEE genome sequence. If only 1/2 of the genome were carried by the VRP, the replicase complex could not be made, and the genome would not self-amplify or express the inserted gene.

Unlike the large DNA viruses (e.g. herpesviruses), the members of the alphavirus genus of the togavirus family contain small RNA genomes comprised of only 7 genes, and the elimination of any one of them would produce a non-viable genome. Such small viruses with RNA genomes are well-represented in the Risk Group 3 or 4 eukaryotic viruses, and some of these have been engineered to produce virus vector systems for use under BL-1 containment. The current regulation has limited this work to those viruses whose replication machinery is encoded by less than 2/3 of their genome. (An arbitrary limit, because the ability to produce viable progeny would likely be lost in a 90% genome.) Changing the requirement so that such vectors must contain 50% or less of the virus genome would, in many cases, reclassify them as BL-3, just as it would VRP.

It would be better to amend the requirement for genome content in a way that takes the biology of the specific virus family into account. Small RNA viruses should not be treated in the same way as large DNA viruses, because they differ dramatically in the proportion of the genome dedicated to basic genome replication.

Robert M. Shanks, Ph.D
Assistant Professor
Department of Ophtalmology

University of Pittsburgh

-----Original Message-----
From: Shanks, Robert M [mailto:shanksrm@upmc.edu]
Sent: Wednesday, April 22, 2009 3:28 PM
To: Office of Biotechnology Activities (NIH/OD)
Subject: antibiotic resistance in the basic laboratory

Dear Office of Biotechnology,
I am writing in response to the "biosafety concerns for research with synthetic DNA" in basic laboratories noted in the Federal Register/Vol. 74, No. 41/Wednesday, March 4, 2009/Notices.

I am against further regulation of generating antibiotic resistant microorganisms in the laboratory. At first this may sound irrational, because the spread of antibiotic resistance is a major concern and an important public health problem. However, placing layers of regulation on the use of antibiotic resistance markers in biology would be a bigger health issue through its negative effects on research.

The use of bacteria in biological science is a fundamental to many research projects, and that is accomplished largely with tools that are used and monitored by their ability to confer antibiotic resistance to the bacteria in use. It is standard practice to destroy the resistant organisms with autoclaves or bleach treatment before disposal to prevent release of these organisms into the environment.

As a basic scientist and molecular microbiologist that works to reduce human and animal infectious diseases caused by bacteria, I must say that the use of antibiotic resistance as a genetic tool in the laboratory is of utmost value. Without the use of antibiotic resistance markers our understanding of the mechanisms of pathogenesis at the molecular level would be almost nothing. Moreover, there are few biological researchers, whether they study chromosome segregation defects or cancer biology, who do not use antibiotic resistance markers on a regular basis. We commonly make non-infectious laboratory strains of E. coli resistant to ampicillin or kanamycin antibiotics for example. This is often to express genes to make proteins for a myriad of purposes, in medicine, industry, and basic science.

Certainly, select agents that can be used for biological warefare should be closely monitored and heavily regulated, but organisms used for day to day protein production or DNA production, such as E. coli, and those studied for their role in infectious diseases, such as Pseudomonas aeruginosa and Staphylococcus aureus should not prevented from being used with antibiotic resistance tools.

Alternatives, including the use of nutritional genes to take the place of antibiotic resistance markers can be done, as it is commonly done with the study of Saccharomyces cerevisiae. However, these alternatives come at a high price, because the organism must first be modified in order to make use of the nutritional genes. Mutation of nutritional genes in the original microorganism can both be daunting in some species, and can cause unexpected physiological effects that confound the interpretation of the study.

The problem with antibiotic resistance microorganisms is much more likely to stem from overuse of antibiotics in agriculture and medicine, than it is with their use in laboratories.

In short, I cannot overemphasize the importance of antibiotic resistance markers in biology. Giving them up would be analogous to giving up the use of electricity or the use of computers. Any large regulatory blocks to their use would have a profound negative impact on our ability to perform biological research and a crippling effect on our ability to compete on an international level.

Best regards,
Robert Shanks, PhD
Assistant Professor
Department of Ophthalmology
University of Pittsburgh
Pittsburgh, PA 15213

Bruce A. McClane
Professor of Microbiology and Molecular Genetics

University of Pittsburgh

The opinions expressed in this memo are those of the author and are not necessarily the official position of the University of Pittsburgh.

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Bruce McClane, Ph.D.
Professor of Microbiology

April 28, 2009

Office of Biotechnology Activities,
National Institutes of Health
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892-7985

Subject: Response to request for comments on the proposed revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register*, 9411-21

The following comments are respectfully submitted in response to the proposed Section III-A-1 revisions to the NIH Guidelines for Research Involving Recombinant DNA Molecules, published in the March 4, 2009 *Federal Register*, 9411-21:

There is justified reason for concerns about increasing antibiotic resistance among bacterial pathogens. However, this emerging problem is due to misuse and overuse of antibiotics in the clinical and agricultural settings rather than a consequence of laboratory research. The current NIH regulations facilitate the routine laboratory research use of naturally-occurring antibiotic resistance markers in a bacterial species, provided those resistance determinants are not front-line therapeutics; this policy has never resulted in documented problems. Thus, the rationale for the proposed revision to Section III-A-1 of the NIH guidelines for recombinant DNA molecules is unclear and concerning for at least two reasons:

1) The use of antibiotic resistance genes as selectable markers is a ubiquitous, essential tool for research on bacterial genetics and virulence. This research involves a large number of different species of bacterial pathogens, each with many different natural drug resistance genes against non-frontline therapeutics. Thus, IBC and RAC committees would likely be overwhelmed by the proposed new oversight responsibilities. The resultant regulatory bottleneck would impede research to improve public health, including the development of new vaccines and therapeutics.

The timing of this proposed new regulation is unfortunate. American bacteriologists have experienced six years of limited NIH funding. Now, just as the NIH budget situation appears to be improving, the proposed regulatory rDNA oversights would be a new hurdle. Unless similar restrictions on research use of "in

species" antibiotic resistance determinants against non-frontline therapeutics are simultaneously instituted by other countries, the regulatory delays imposed by implementing the new rDNA rules would once again leave American scientists at a competitive disadvantage. In the long term, this handicap would negatively impact the American economy as vaccine and therapeutic development would become less burdensome outside the USA.

The delays associated with these proposed regulatory rules would also impact progress on NIH grants. NIH would get less return on their research investment if scientists must spend even more time completing paperwork needed to comply with the new regulations and waiting for IBCs and RAC responses to these protocols. This would likely be a continual, ongoing process for most investigators, who use different antimicrobial resistance determinants for numerous purposes, e.g. selection of targeted mutagenesis vectors, random mutagenesis vectors, vectors for expression of recombinant proteins and complementation, etc.

2) The proposed additional regulation of antibiotic resistance traits would not materially impact/limit the spread of existing antibiotic resistance traits within a bacterial species, which is the apparent main intent of these new rules. These antibiotic resistance genes are naturally present on mobile genetic elements, such as conjugative plasmids and transposons, and thus readily move within the population of any particular species in the natural environment.

Considering the above points, and the very questionable need for new regulations, NIH should reconsider this proposed change and, instead, continue the current regulations that have worked extremely well for many years.

Sincerely,

Bruce A. McClane

Bruce A. McClane,
Professor of Microbiology and
Molecular Genetics