406 of the Food and Drug Administration Modernization Act of 1997 (21 U.S.C. 393), which include working closely with stakeholders and maximizing the availability and clarity of information to stakeholders and the public. This is consistent with the purposes of ORA's Small Business Representative Program, which are in part to respond to industry inquiries, develop educational materials, and sponsor workshops and conferences to provide firms, particularly small businesses, with firsthand working knowledge of FDA's requirements and compliance policies. The workshop is also consistent with the Small Business Regulatory Enforcement Fairness Act of 1996 (Public Law 104–121), as outreach activities by government agencies to small businesses.

The goal of the public workshop is to present information that will enable manufacturers and regulated industry to better comply with labeling requirements, especially in light of growing concerns about obesity and food allergens. Information presented will be based on agency position as articulated through regulation, compliance policy guides, and information previously made available to the public. Topics to be discussed at the workshop include the following: (1) Mandatory label elements, (2) the Food Allergen Labeling and Consumer Protection Act of 2004, (3) nutrition labeling requirements, (4) health and nutrition claims, and (5) special labeling issues such as exemptions. FDA expects that participation in the public workshop will provide regulated industry with greater understanding of the agency's regulatory and policy perspectives on food labeling and increase voluntary compliance with labeling requirements.

Dated: April 16, 2010.

Leslie Kux,

Acting Assistant Commissioner for Policy.

[FR Doc. 2010–9288 Filed 4–21–10; 8:45 am]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Office of Biotechnology Activities; Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

AGENCY: National Institutes of Health (NIH), PHS, DHHS.

ACTION: Notice of a proposed action under the *NIH Guidelines.*

SUMMARY: In March 2009, the NIH Office of Biotechnology Activities (OBA) published a proposal to revise the *NIH Guidelines for Research with Recombinant DNA Molecules (NIH Guidelines)* to address biosafety for research with synthetic nucleic acids (74 FR 9411). The proposal included amending the scope of the *NIH Guidelines* to specifically encompass research with synthetic nucleic acids. In addition, in consultation with the NIH Recombinant DNA Advisory Committee (RAC), OBA proposed changes to several other sections of the *NIH Guidelines,* including Section III–E–1, which addresses containment for work with partial viral genomes in tissue culture. In response to public comments received on the proposed changes to Section III–E–1 (74 FR 9411), a substantively revised proposal has been developed and OBA is seeking additional comment on this Section. After comments are received on this revised proposal and reviewed at a public RAC meeting, OBA will publish a final notice of action for Section III–E–1 and the other proposed revisions included in the March 2009 **Federal Register** (FR) notice.

Section III–E–1 of the *NIH Guidelines* allows investigators to proceed with certain tissue culture experiments under Biosafety Level 1 (BL1) containment upon registration of the experiment with an Institutional Biosafety Committee (IBC). Under the current *NIH Guidelines,* an investigator can initiate an experiment in tissue culture at BL1 containment if no more than two-thirds of the full viral genome is present and the preparation is free of "helper virus," *i.e.*, a virus that could be used to rescue infectious, replication competent virus. Experiments performed under III–E–1 apply to viruses in all Risk Groups except for *Variola major or Variola minor* (smallpox, alastrim, whitepox—Section III–D–3–d). In the March 2009 FR, OBA proposed to reduce the portion of the genome that could be present to less than one-half due to concerns that synthetic techniques might lead to functional viruses that contained less than two-thirds of a full viral genome. Based on the comments received in response to the FR notice of March 2009, discussions at a public stakeholder meeting on June 23, 2009 [see URL: *http://oba.od.nih.gov/rdna_rac/rac_pub_con.html*] and further consultations with the RAC, OBA is amending its original proposal to include additional criteria for lowering containment. These new criteria will allow containment to be lowered to BL1 for experiments performed in tissue culture when more than one-half of the genome is present, as long as the function of critical viral genes is sufficiently understood to allow the determination that a complete deletion in one or more essential viral capsid, envelope or polymerase genes required for cell-to-cell transmission of viral nucleic acids will effectively impair viral replication. The deletion(s) must be designed such that it is not possible to rescue critical functions through homologous recombination. If such a deletion is not feasible or practical, an experiment may also be included under Section III–E–1 if the recombinant viral genome contains less than one-half of the full viral genome. As explained in the March 2009 proposal, this latter criterion would only apply to Risk Group (RG) 3 and RG4 viruses (*see NIH Guidelines* Appendix B) as the *NIH Guidelines* currently exempt research with less than one-half of the genome of RG1 or RG2 virus (*NIH Guidelines* Appendices C–I and C–I–A).

In light of this substantive change from the original proposal, OBA is seeking further comment on this revised proposal.

DATES: The public is encouraged to submit written comments on this proposed action. Comments may be submitted to OBA in paper or electronic form at the OBA mailing, fax, and e-mail addresses shown below under the heading **FOR FURTHER INFORMATION.** All comments should be submitted by June 1, 2010. All written comments received in response to this notice will be available for public inspection in the NIH OBA office, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892–7985, weekdays between the hours of 8:30 a.m. and 5 p.m.

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

SUPPLEMENTARY INFORMATION:

Background: Section of III–E of the *NIH Guidelines* addresses experiments for which IBC notification is required at the time the research is initiated. Experiments covered in this section of

the *NIH Guidelines* are considered to be of low biosafety risk and therefore although IBC review and approval is still required, such approval need not be obtained prior to initiating research. This is in contrast to all other experiments described in the *NIH Guidelines* for which IBC review and approval is required prior to initiation of the experiment.

Section III–E–1 of the *NIH Guidelines* addresses biocontainment levels for experiments involving eukaryotic viruses propagated and/or maintained in tissue culture systems. The current language in the *NIH Guidelines* allows the experiment to be conducted under BL1 containment provided that a given recombinant DNA molecule contains no more than two-thirds of the genome of a eukaryotic virus from the same Family ("the two-thirds rule"). 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."

Thus to qualify for a reduction in containment pursuant to this section, the recombinant molecule may be constructed from (1) 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 or (2) the recombinant molecule may be constructed from genomic fragments of viruses from different taxonomic Families provided that each fragment from a single viral Family used in the construct conforms to the two-thirds rule. In addition, it must be demonstrated that the tissue culture system is free of helper virus that could lead to rescue of infectious virus. If helper virus is present, containment is determined by Section III–D of the *NIH Guidelines.* Under Section III–D containment is usually determined by the RG designation for the eukaryotic virus.

This section was reviewed by the RAC in response to concerns that it may be possible to construct, using synthetic methods, a virus that would contain less than two-thirds of the genome of any one virus or Family of viruses but still be potentially infectious. In addition, in light of current understanding of virus biology, it was proposed that it might be possible to develop a criterion based on deletion of functional genes in lieu of a quantitative genome percentage. The RAC also recognized that the requirement to demonstrate the absence of helper virus did not address other ways in which infectious virus could be rescued. For example, it has been demonstrated that replication competent adenovirus can arise from HEK 293 producer cells via homologous recombination in the absence of any helper virus; [1] similar events have also been reported in murine retrovirus producer cells.[2]

After discussion of several potential criteria to define what constitutes a functionally defective virus, the RAC ultimately recommended retaining a quantitative threshold. In part, this was due to the need for an unambiguous standard, as this section allows investigators to initiate experiments at the lowest level of containment (BL1) prior to IBC review and approval. The RAC recommended that OBA consider changing the two-thirds rule to a "one-half rule," such that one could only initiate these experiments in tissue culture when less than one-half of the full viral genome was present. This was based in part on concerns that novel approaches to genetic manipulation could lead to the creation of novel minimal genomes [3] while maintaining viability at least under in vitro conditions.

Based on these recommendations, OBA proposed the following changes to Section III–E–1 in the March 4, 2009 **Federal Register** notice:

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 acids 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 from any given Family shall be less than one-half of a genome.

Comments Submitted in Response to the March 2009 FR Notice

Five comments were submitted to OBA in response to the proposed revisions to Section III–E–1 of the *NIH Guidelines.* All of these comments focused on the proposal to require that only one-half of the genome be present instead of the previous two-thirds. Two comments questioned the validity of limiting the applicability of Section III–E–1 to RG3 and RG4 viruses without inclusion of RG2 viruses. No change was made in response to these comments because the *NIH Guidelines* currently exempt tissue culture experiments involving RG1 and/or RG2 viruses in which more than one-half of their genome are deleted (*see* Appendices C–I/C–I–A).

Two comments agreed with the proposed revisions but two other comments questioned the validity of stipulating a relative genome size (*i.e.,* less than one-half) as the basis for lowering containment rather than relying on the inability of a virus to replicate, regardless of the amount of viral genomic sequence effectively deleted. One of these comments further expressed significant concerns about the impact that the proposed revisions would have on ongoing recombinant

[1] Lochmüller, H., *et al.* (1994). Emergence of early region 1-containing replication-competent adenovirus in stocks of replication defective adenovirus recombinants (ΔE1 + ΔE3) during multiple passages in 293 cells. *Hum. Gene Ther.* 5:1485–91.

Hehir, K. *et al.* (1996). Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. *J. Virol.,* 70(12):8459–67.

Fallaux, F. J., *et al.* (1998). New helper cells and matched early region 1-deletion adenovirus vectors prevent generation of replication-competent adenoviruses. *Hum. Gene Ther.,* 9:1900–17.

[2] Otto E., *et al.* (1994). Characterization of a replication-competent retrovirus resulting from recombination of packaging and vector sequences, *Hum Gene Ther.* 5:567–75.

[3] Lartigue, C. *et al.* (2009). Creating Bacterial Strains from Genomes That Have Been Cloned and Engineered in Yeast. Science 325(5948):1693–96.

Hutchison III, C.A. *et al.* (1999). Global transposon mutagenesis and a minimal mycoplasma genome. Science 286(5447):2165–9.

research involving Venezuelan Equine Encephalitis virus (VEE), a virus of the Family *Togaviridae,* Genus *Alphavirus.* The comment noted that ongoing research on defective viral replicon particles (VRP) of VEE has been supported by NIH funding for at least 15 years and that these defective genomes contain less than two-thirds but more than one-half of the viral genome. VRP-based vaccines are currently under evaluation in clinical trials. The central feature of VRP-based vaccines is their ability to express an inserted non-VEE gene at high levels for induction of an immune response in the absence of all viral replication. The essential viral components encoded within the missing one-third of the defective VEE genome sequence are all the capsid structural components; these are required for infectious particle formation and virus replication. Removing more than one-half of the VEE genome from VRP particles would disable the essential viral RNA replication machinery that is key to the high level VRP expression system, and constitutes the functional basis of the vaccine itself. The comment went on to note that a number of RG3 and RG4 viruses contain a small number of genes, and elimination of any one of them produces a non-viable virus. It is thus possible to disable certain viruses by deleting far less than one-third of their genome.

OBA considered these comments carefully with input from the RAC. The comment about RG3 and RG4 viruses led to further discussion of whether there were certain types of genes that, if deleted, would consistently produce severe functional deficiencies such that virus replication would be completely or sufficiently impaired to ensure the loss of transmissibility and infectivity. The proposed language presented herein would allow experiments using viral constructs (excluding all research with *V. major* or *V. minor*) that contain targeted genomic deletions, which impair the ability of the virus to replicate in tissue culture, to be conducted at BL1 containment under Section III–E–1. The proposed language specifies both the type of impairment (*i.e.,* deletion) and the biological targets for these impairments (capsid, envelope or polymerase genes, *i.e.,* functions critical for cell to cell transmission). As many tissue culture experiments are routinely carried out at BL2 containment to avoid contamination of the culture, this section primarily allows containment to be lowered for work with RG3 and RG4 viruses. The majority of RG3 and RG4 viruses are RNA viruses. The structural genes listed above are the favored functional targets historically used to genetically disable these higher risk group viruses under the existing "two-thirds rule." If sufficient knowledge about the function of particular viral genes exists, it will now be possible to impair the virus through targeted deletions and to qualify for containment reduction regardless of the quantity of the genome that is deleted. However, a complete deletion of genetic sequence will be required such that it will not be possible to rescue biological function by homologous recombination among partial viral genomes or nucleic acids present in tissue culture cells used for virus or vector rescue. Therefore, this new criterion should still ensure that only work that can be safety conducted at BL1 will be allowed to proceed.

This criterion would be in addition to the one-half rule that was proposed in the March 2009 FR notice. The RAC recommended retaining a quantitative threshold of one-half a genome size for those viruses in which the understanding of the biology of the virus is incomplete and therefore it is not possible to predict with certainty the effect that any particular genetic impairment will have on the ability of a virus to replicate and infect cells. Again, the latter will only apply to RG3 or RG4 viruses in tissue culture as experiments with recombinant molecules containing less than one-half of the genome of RG1 or RG2 agents are currently exempt under the *NIH Guidelines.*

Finally, OBA notes that while most tissue culture experiments will be performed at BL2, Section III–E–1 as proposed does permit containment to be lowered to BL1. However, concerns were raised regarding risks associated with integrating viruses that could cause insertional mutagenesis. Appendix B–V of the *NIH Guidelines* states that for some animal agents that are infectious to human cells, *e.g.,* amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 agents is recommended. In addition, in 2006, OBA issued a *Guidance on Biosafety Considerations for Research with Lentiviral Vectors* (*http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html*) that also recommended a minimum of BL2 for most research with lentiviral vectors. In light of these requirements, OBA has clarified that BL2 containment should be used for tissue culture experiments using retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

OBA is requesting comment on the following proposed revision to Section III–E–1:

Section III–E–1. Experiments Involving the Formation of Recombinant DNA Molecules Propagated and Maintained in Tissue Culture Systems

Recombinant nucleic acids from a eukaryotic virus (excluding all research with *V. major* or *V. minor*) and/or synthetic nucleic acid molecules based on a sequence from a eukaryotic virus (excluding *V. major* or *V. minor*) may be propagated and maintained in cells in tissue culture using BL1 containment (as defined in Appendix G) if:

(i) There is a complete deletion in one or more essential viral capsid, envelope or polymerase genes required for cell-to-cell transmission of viral nucleic acids or

(ii) For Risk Group 3 or Risk Group 4 viruses no more than half of the genome is present, (all viruses from a single Family being considered identical [*see* Section V–J, *Footnotes and References of Sections I–IV*]). 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.

In addition, there must be evidence that the resulting nucleic acids are not capable of producing a replication competent virus in a cell line that would normally support replication of the wild-type virus. When reduction in containment is based on a deletion in one or more essential viral capsid, envelope or polymerase gene, evidence such as sequence or other appropriate data, should be submitted to the IBC to demonstrate that there is a complete deletion of genetic sequence such that these functions can not be rescued through homologous recombination. It must also be demonstrated that the cells lack helper virus for specific Families of defective viruses being used. If helper virus is present, review will proceed 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.*

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

Dated: April 16, 2010.

Jacqueline Corrigan-Curay,

Acting Director, Office of Biotechnology Activities, National Institutes of Health.

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