BEGINNER’S GUIDE TO THE NIH GUIDELINES

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) are a set of regulations that specify practices for the safe handling of recombinant DNA (rDNA), synthetic (sDNA) molecules and the organisms that contain rDNA molecules. Even though they are called guidelines, they are legally binding for any institution using NIH funds. This guide is designed to provide an introduction to the NIH Guidelines. The full NIH Guidelines should be consulted when necessary and can be found at <http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>. Plant, whole animal, and human experimentation are discussed in special sections of the NIH Guidelines.

***What are recombinant DNA (rDNA) and synthetic DNA (sDNA)?***

The NIH Guidelines (Section I-B) define recombinant and synthetic nucleic acids as follows:

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

(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or

(iii) molecules that result from the replication of those described in (i) or (ii) above.

**If your work involves the use of rDNA or sDNA, we recommend that (1) you read this entire document, (2) you read section III of the NIH Guidelines and (3) you complete an rDNA/infectious agent application available on line.**

***What are the RAC and the OBA?***

The Office of Biotechnology Activities (OBA) is an administrative arm of the National Institutes of Health responsible for carrying out the orders of the NIH Director with regard to rDNA, genetic testing and xenotransplantation. An advisory committee is involved in establishing policies for each of these fields. For rDNA, the committee is called the Recombinant DNA Advisory Committee or "RAC”.

***Authority of the MU IBC***

The Marshall University (MU) Institutional Biosafety Committee (IBC) serves to insure that the NIH Guidelines are followed by all investigators and to review rDNA registrations. The IBC reports to Dr. John Maher who is the VP for Research. Questions about the Guidelines or IBC policy can be addressed to Donald A. Primerano, IBC, Chair or to Nathan Douglas, Biological Safety Officer.

The IBC committee is guided by and ensures compliance with the following:

• the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and Gene Transfer (“NIH GUIDELINES”) which specifies practices for the construction, handling, and disposal of recombinant and synthetic DNA,

• the CDC Select Agent Program (42 CFR Part 73) which governs the possession, use, and transfer of select infectious agents and toxins,

• the Joan C. Edwards School of Medicine (JCESOM) Bloodborne Pathogen Exposure Control Plan which addresses:

o (1) risk classification for each SOM job at the Byrd Biotechnology Science Center and Medical Education Building,

o (2) implementation of methods of compliance with regulations and policies,

o (3) Hepatitis B vaccination (or waivers) and post-exposure follow-up,

o (4) communication of hazards to employees through training, signs and labels,

o (5) procedures for evaluating circumstances surrounding exposure incidents and

o (6) special rules for HIV/HBV research or production facilities.

• BBSC and TGRI Infectious Waste Management Plan whose objectives are to provide

o (1) a safe and controlled environment for visitors, students, faculty and staff and

o (2) proper management of infectious waste in accordance with the West Virginia Infectious Waste Rule 64-CSR-56 and the Occupational Safety and Health Administration health regulations on exposure to Blood Borne Pathogens from its receipt by the University until its appropriate destruction or removal, and the

• Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual (DHHS/CDC).

***rDNA REGISTRATION PROCESSES***

All non-exempt rDNA and sDNA research must be approved by the IBC and in some cases by NIH level administration. Section III of the NIH Guidelines describes six categories of experiments involving recombinant or synthetic nucleic acid molecules and explains which approvals are needed:

(i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see Section III-A), “**Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally”.** Work cannot begin until there is RAC review, NIH Director approval and IBC approval.

(ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see Section III-B), **“Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms per Kilogram Body Weight” and “ Approved Major Actions”.** Work cannot begin until there is NIH/OBA approval and IBC approval.

(iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see Section III-C), **“Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants”.** Work cannot begin until there is RAC review, IBC approval and IRB approval.

(iv) those that require Institutional Biosafety Committee approval before initiation (Section III-D),

**D-1: Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See Section II-A, Risk Assessment)**

**D-2: Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector SystemsD-3: Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems**

**D-4: Experiments Involving Whole AnimalsD-5: Experiments Involving Whole Plants**

**D-6: Experiments Involving More than 10 Liters of Culture**

**D-7: Experiments Involving Influenza Viruses**

Work cannot begin until there is IBC approval.

(v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-E):

**“Experiments not included in Sections III-A, III-B, III-C, III-D, III-F, and their subsections are considered in Section III-E. All such experiments may be conducted at BL1 containment.” *At MU,* t*he IBC Chair must still review these protocols to insure that they fall under Section III-E.***

(vi) those that are exempt from the NIH Guidelines (see Section III-F). There are currently eight subcategories of exempt research (III-F-1 through III-F-8).

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

Section III-F-2. Those that are not in organisms, cells, or viruses and that have not been modified or

manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.

Section III-F-3. Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a

single source that exists contemporaneously in nature.

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

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

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

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

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

***Application Submission Process***

**The MU IBC requires all labs working with rDNA or sDNA to request approval for their work by submitting an rDNA/sDNA/infectious agent application form (available at the IBC website). This form must be completed submitted for rDNA/sDNA even if the work falls into the exempt category. An investigator may request exempt status as part of the application process. If the IBC chair concurs, the chair will send a letter of approving the work and declaring it as exempt. It the IBC chair disagrees and finds that the work is non-exempt, then the entire application must be reviewed by the IBC.**

***Biosafety levels and corresponding work practices***

There are four levels of biosafety that specify sets of practices needed for the safe handling of rDNA, sDNA and pathogenic organisms. Biosafety Levels 1 through 4 (BL1 through BL4; also known as BSL-1 through BSL-4).

***Risk Groups***

The NIH classifies biological agents into four Risk Groups according to their human pathogenicity (see NIH Guidelines, Section II-A-1). In considering pathogenicity, individual and community risks are taken into account:

o Risk Group 1 - not associated with disease in healthy adults.

o Risk Group 2 - associated with disease that is rarely serious and for which therapeutic or

preventive options are *often* available.

o Risk Group 3 - associated with serious or lethal disease for which therapeutic or preventive options *may* be available.

o Risk Group 4 - associated with serious or lethal disease for which therapeutic or preventive options are *not usually* available.

For more information about safety practices and precautions consult the Summary of Recommended Biosafety Levels for Infectious Agents in the BMBL (Table 2, p 59). <http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf>

***Specialized Sections of the Guidelines***

The NIH Guidelines recognize three non-laboratory classes of Biosafety containment and procedures; those in which genes are transferred into humans (Appendix M); those for plants (BL1- P through BL4-P, Appendix P) and those for animals (BL1-N through BL4-N, Appendix Q).

o **HUMANS**

All Human Gene Transfer protocols are currently considered experimental. The IBC may establish a Human Gene Therapy Advisory Committee to deal with human Gene Transfer studies. IBC approval must await RAC (NIH Recombinant Advisory Committee) action. Depending on whether the study is deemed “novel” the RAC can either schedule a full examination of the protocol at one of its quarterly meetings or recommend sole FDA review.

Beyond approvals from the Food and Drug Administration one has to get approval from the local Institutional Review Board and the IBC. In addition, the RAC will evaluate novel protocols although it does not have approval power. These evaluations often involve the PI's appearance at a RAC meeting with questioning by the RAC panel.

o **ANIMALS**

Animal Biosafety levels are normally used to cover large animals such as cattle, swine, horses, and poultry. The IBC tends to use the same designations when considering safe practices with smaller animals including rodents.

All animal experiments must be reviewed and approved by the MU Institutional Animal Care and Use Committee (IACUC). If rDNA or sDNA are involved, IBC approval is required at a minimum.

o **PLANTS**

Plant Biosafety levels are necessary when research plants are too big, too many or have growth requirements that cannot be covered by the standard Biosafety Levels. The plant guidelines cover plant associated microorganisms and insects. Plant associated microorganisms include viroids, bacteria, viruses, fungi, protozoans, as well as benign or beneficial microorganisms known to be associated with plants. When studies covered under the plant appendix are being discussed the IBC will include an expert in plant pests or containment.

It is of interest that the plant guidelines are not designed to directly protect humans from plant related recombinant DNA. The agents covered pose virtually no threat to humans or higher animals. Rather the guidelines are in place to protect the general ecosystem from serious disruption. Thus procedures are designed to limit the spread of novel organisms from the experimental facility, not to protect the workers.