THE NIH RECOMBINANT DNA (rDNA) GUIDELINES EXPLAINED

(April 2002 Version)

The next few pages were written to extract the essence of the NIH Guideline requirements and put them into readable form. Since many details are omitted (and the devil is in the details), the actual Guidelines should be consulted when in doubt (http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html). Plant, whole animal, and human experiments are given special treatment in the guidelines (and in this explanation). The NIH recombinant DNA Guidelines are an intimidating publication. They are long, full of legalese, and have lots of cross references, exceptions, lists, sections, appendices, and tables. No one actually reads the Guidelines. But they are legally binding on any institution using NIH funds.

PREREQUISITES

To make sense of the following, the reader should know that the UCHC Institutional Biosafety Committee is called the IBC. The IBC may be contacted through the Office of Research Safety (ORS) – the Biological Safety Officer (BSO) is the coordinator of the IBC. Also periodically check the ORS website.

The reader should also know the basic ideas behind Biosafety Levels 1 through 4 (BL1 through BL4; also known as BSL-1 through BSL-4). Summary tables giving the characteristics of the four laboratory Biosafety Levels and Animal Biosafety Levels are on the last two pages .

REGISTRATION = SUBMISSION OF A REGISTRATION DOCUMENT (REG. DOC.) TO THE IBC.

At a minimum, the UCHC IBC requires all labs working with rDNA to inform the IBC about what they are doing that involves rDNA. The Guidelines distinguish among four types of IBC approved registrations and one IBC determination of exemption. The types depend on the potential hazard; the more hazard, the more approvals are required. The 4 types are:

- Work that cannot begin until there is NIH and IBC approval (Sections III-A and III-B), this is the most dangerous level and it is **[TABOO]** (page 2).
- Work that cannot begin until there is IBC approval and RAC review (Section III-C), this tends to be sensitive and potentially dangerous. See **[WAIT AND WAIT],** page 2. *Human Gene Transfer Studies* are in this category (page 5). This *example* also requires IRB approval.
- Work that cannot begin until there is IBC approval (III-D). There is usually a **[WAIT]** (page 2). *Human Xenotransplantation Studies* are in this category (FDA requirement).
- Work that can begin as soon as the reg. doc. is accepted by the IBC (Section III-E) **[MINOR WAIT]** (page 4). Acceptance means it makes sense to the screener (BSO).
- If work is Exempt from NIH guidelines (Section III-F), no approval is needed, but the IBC must be informed about all projects, which are then categorized by the BSO **[ALMOST NO WAIT]** (page 4). Submit no more than one page describing all host-vector systems and strains used per experiment.

WHAT IS RECOMBINANT DNA?

It is either a) DNA constructed *in vitro* from separate DNA segments that can replicate and/or express a biologically active polynucleotide or polypeptide *in vivo*, or b) synthetic DNA that has the potential of generating a hazardous product *in vivo*.

WHAT ARE THE RAC AND THE OBA?

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

“TABOO”
(NIH APPROVAL AND IBC PERMISSION REQUIRED)
(from NIH Guidelines Sections III-A and III-B)

- Making drug resistant constructs of microorganisms if they compromise the drug’s therapeutic potential,
- Making constructs that synthesize vertebrate toxins with an LD50 of 100 ng/Kg or less,
- Making constructs except in *E. coli* K-12 that synthesize vertebrate toxins that are “lethal” between 100 ng/Kg and 100 µg/Kg (check the specifics in the Guidelines, Section III-B-1).

“WAIT AND WAIT”
(NIH REVIEW AND IBC PERMISSION REQUIRED)
(from Section III-C)

Studies in which genes are transferred into humans must be submitted to the NIH OBA for review. If the OBA find the study to be "novel" it will place the study on the next RAC meeting agenda. IBC approval must wait for the RAC's review. If, on the other hand, OBA does not deem the study to be "novel," the IBC can act immediately. When the UCHC is an additional IND trial site, the RAC review for the study may already exist.

“WAIT”
(IBC APPROVAL NEEDED BEFORE STARTING)
(from Section III-D)

Most studies carried out at UCHC laboratories are in this group. They are examined by IBC with an eye to recommending safe procedures and containment. To reach a conclusion it is often useful to classify the risk associated with a proposed study according to the risk associated with the organisms to be used. A convenient classification tool is the concept of “risk group.”

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:

- Risk Group 1 - not associated with disease in healthy adults.
- Risk Group 2 - associated with disease that is rarely serious and for which therapeutic or preventive options are *often* available.
- Risk Group 3 - associated with serious or lethal disease for which therapeutic or preventive options *may* be available.
- Risk Group 4 - associated with serious or lethal disease for which therapeutic or preventive options are *not usually* available.

Appendix B in the NIH Guidelines lists a number of biologic agents according to their Risk Group.

In general, the Risk Group determines the Biosafety Level needed: for instance, a Risk Group 3 agent is usually studied in a BL3 or BL3-N (animal) lab. Note that at present (2003), the UCHC has no facilities above BL2.

The table on the next page summarizes the NIH recommendations for Biosafety Level according to risk group.

• Gene Transfer into Human subjects (RAC review process[1] is required before the IBC can approve this kind of study). (Section III-C-1)		
• Recombinant organisms cultured in volumes greater than 10 liters require IBC approval (Section III-D-6).		
• Pathogen Hosts (Section III-D-1): Check Appendix B to determine their Risk Group - then:		
	Risk Group 2 host	BL2, BL2-N
	Risk Group 3 host	BL3, BL3-N
	Risk Group 4 host	BL4, BL4-N
• Pathogen DNA source into Non-Pathogen Host (Section III-D-2): Check Appendix B to determine their Risk Group - then:		
	Risk Group 2 or 3 source	BL2
	Risk Group 4 source	BL2, **IF** the pathogen genome is defective
	Risk Group 4 source	BL4, **Otherwise**
• Animal Virus DNA source into Tissue Culture (Section III-D-3): Check Appendix B to determine their Risk Group - then:		
	Risk Group 2 virus source	BL2
	Risk Group 3 virus source	BL3
	Risk Group 4 virus source	BL4
• Transgenic *Animal* [2] Host (Section III-D-4):		
	Anything but > 2/3 eukaryotic virus genome	BL1-N (but IBCcan boost this level based on the pathogenicity of the source organism)
	Viral vectors that don't transmit	BL1-N
	Everything else is a special case	the IBC decides
• Modified microorganisms into *Animals*: (Section III-D-4)		
	Any viable rDNA modified organism	≥BL2, BL2-N
• rDNA into *Animals (*Section III-D-4-a, see this section in the Guidelines)		
	Any rDNA (except pieces with >⅔ eukaryotic viral genome)	BL1, BL1-N
	Everything else	the IBC decides
• Whole *Plants*: (Section III-D-5)		
	Exotic [3] pathogen hosts that can damage the ecosystem	BL3-P or BL2-P+
	Plants with transmissible DNA from exotic pathogens that can damage the ecosystem	BL3-P or BL2-P+,
	Transmissible exotic pathogen hosts	BL4-P
	Toxin DNA into plants	BL3-P
	Insect Pathogen DNA that can damage the ecosystem	BL3-P or BL2-P+
• Genes coding for vertebrate **Toxins** (Appendix F)		
	LD_{50} < 100 ng/Kg	Requires NIH & IBC approval
	100 ng/Kg < LD_{50} < 100 µg/Kg	Requires IBC approval & NIH notification. (except in E. coli - see below)
	100 ng/Kg < LD_{50} < 1µg/Kg	BL2 if in E. coli (K - 1 2)
	1 µg/Kg < LD_{50} < 100 µg/Kg	BL1 if in E. coli (K - 1 2)

[1]RAC can either take no action and transmit the protocol to the FDA or call for a full public review at one of its quarterly meetings.

[2]The purchase or transfer of transgenic rodents is exempt from the NIH guidelines.

[3]"Exotic" plant pathogens are defined as those not known to occur naturally in the US.

"MINOR WAIT"
(IBC ACCEPTS REGISTRATION DOCUMENT BEFORE WORK STARTS)
"LOW HAZARD" RECOMBINANT DNA (from NIH Guidelines Section III-E)

All non-exempt recombinant DNA studies (including these low hazard studies) must be registered with the IBC; only the timing of IBC approval is relaxed for low hazard rDNA experiments. Examples of these experiments are:

- On-site construction of transgenic rodents that require BL1 containment,
- Recombinant DNA with < 2/3 to 1/2 (< 1/2 may be exempt) of a eukaryotic viral genome (with no helper or replication competent recombinant demonstrated) used exclusively in tissue culture (use BL1),
- Experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes are under this section (use BL1),
- For experiments with *Plants* carrying recombinant DNA or plant associated microorganisms or small animals, see below (Specialized Guideline Sections, page 5), and/or contact the BSO.

"ALMOST NO WAITING"- Exempt Recombinant DNA Experiments
(IBC ACCEPTS EXPLANATORY DOCUMENT BEFORE WORK STARTS)
(from NIH Guidelines Section III-F and Appendix C)

- "Exempt" from NIH guidelines means that work with these constructs doesn't need to be approved by the IBC. However, the determination of what recombinant DNA work is exempt or non-exempt is often complicated. Thus, *it is the IBC's policy to insist that investigators using any rDNA must explain their work to the IBC or BSO* so that a determination of exempt or non-exempt status can be made. Submit one page describing all host-vector systems and strains used per experiment.

SOME EXEMPT CLASSES OF WORK ARE EXPERIMENTS WITH OR EXPERIMENTS WHERE:

- Recombinant DNA outside of living organisms or viruses,
- Recombinant DNA that cannot replicate or express *in vivo*,
- DNA from a single nonchromosomal or viral source,
- The DNA source organism and the host organism are the same organism,
- The DNA source organism and the host organism normally exchange DNA (organisms that are considered to normally exchange DNA are listed in Appendix A),
- The DNA that does "not present a significant risk to health or the environment...", as listed in Appendix C:
    - The recombinant DNA is used exclusively in tissue culture and has < 1/2 eukaryotic viral genome. *There are other exceptions to this rule (Appendix C-I-A). Check with the BSO,*
    - Experiments using an *E. coli* K-12 host-vector system in which the host does not contain conjugation proficient plasmids. (Note: strain BL21 is not a K-12 strain.) There are some restrictions on the vectors used (Appendix C-II, C-II-A). BL1 containment is suggested,
    - Experiments with *Saccharomyces cerevisiae* and *S. uvarum* host-vector systems. There are some restrictions (Appendix C-III, C-III-A). BL1 containment is suggested,
    - Experiments with *Bacillus subtilis* or *B. licheniformis* host-vector systems and in which reversion to spore formation is < 10^{-7}. There are some other restrictions (Appendix C-IV, C-IV-A). BL1 containment is suggested.
- The domestic purchase or transfer of transgenic rodents (e.g., not constructed at UCHC) for experiments that require BL1 containment are exempt from the NIH rDNA Guidelines.

IT IS IMPORTANT TO UNDERSTAND THAT AN EXPERIMENT MAY BE BL1, BUT MAY STILL NEED TO HAVE A REGISTRATION DOCUMENT APPROVED BY THE IBC FOR COMPLIANCE WITH THE NIH rDNA GUIDELINES. ALSO, WHETHER OR NOT THE EXPERIMENT ITSELF IS NIH FUNDED, FUNDING FOR ALL NIH FUNDED PROJECTS AT THE UCHC INVOLVING rDNA MAY BE WITHDRAWN WITHOUT SUCH COMPLIANCE. (See the NIH rDNA Guidelines, Section I-D (including I-D-1 and I-D-2.)

The main roles of the Principal Investigator (PI) and Institution with regard to compliance to the NIH rDNA Guidelines are:

- The role of the PI: "On behalf of the institution, the PI is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research (Section IV-B-7)."
- The role of the Institution: "Assist and ensure compliance with the *NIH Guidelines* by PIs conducting research at the institution as specified in Section IV-B-7 (Section IV-B-1-g)."

More specific responsibilities of PIs and institutions are listed in the Guidelines under the respective sections noted above.

THREE SPECIALIZED GUIDELINE SECTIONS

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).

- **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.

Overcoming the regulatory hurdles involved in gaining approval for a human gene transfer study is not a task for the faint of heart. 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 NIH Recombinant DNA Advisory Committee (the RAC) will evaluate novel protocols although it does not have approval power. These evaluations often involve the PI's appearance in Bethesda and aggressive questioning by members of the RAC.

For most people this process can be intimidating. It may be best to get help early either through a commercial sponsor or a consultant.

Once approved by everyone, the PI still has to make regular reports to the NIH. Within 20 working days of the first subject enrollment, a report is due (see Appendix M -I-C-1). At the one year anniversary of the FDA's IND approval a report similar to the FDA annual report is to be sent to the NIH (see Appendix M-I-C-3). Finally, fatal or life-threatening unexpected Serious Adverse Events that *may* be associated with the gene transfer product must be reported to the NIH within 7 calendar days (see Appendix M-I-C-4).

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 a local Institutional Animal Care and Use Committee (IACUC or “ACC”, here). This committee acts under US Department of Agriculture and DHHS regulations.

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 small “animals," particularly insects, such as arthropods. Plant associated microorganism include viroids, virusoids, bacteria, viruses, fungi, protozoans, as well as benign or beneficial microorganisms known to be associated with plants (e.g., *Rhizobium*).

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.

NOTE: This document was adapted with permission from "The Guidelines Explained" by Dr. Andrew G. Braun. Both the original and the adaptation are meant to orient the reader to reading the "NIH Guidelines for Research Involving Recombinant DNA Molecules" (NIH Guidelines), which should be consulted (well) before initiating research using recombinant DNA (kits and commercial vectors included). This adaptation does not substitute for the NIH Guidelines.

SUMMARY OF LABORATORY BIOSAFETY LEVELS

Biosafety Level	Risk Group	Practices and Techniques	Safety Equipment	Examples
BL1 **Basic Laboratory**	Individual risk: LOW Community risk: LOW	Standard Microbiological Practices.	None: primary containment provided by adherence to standard lab practices during open bench operations.	*E. Coli* K12, Continuous culture of cell lines, e.g., short term, long term culture of most non-primate mammalian tissue.
BL2 **Basic Laboratory** with Biosafety cabinets and other physical containment devices as required.	Individual risk: MODERATE Community risk: LOW	*Level 1 practices plus*: lab coats, autoclaving all biological waste preferred, limited access, biohazard warning signs on doors and equipment.	Partial containment (i.e., Class I or II Biosafety cabinets for procedures which produce aerosols.	Hepatitis B Virus, *Salmonella typhi*, Short term, long term culture of human tumor cell lines, culture of lymphoid lines carrying inducible EBV, many common human pathogens.
BL3 **Containment Laboratory** with special engineering and design features.	Individual risk: HIGH Community risk: MODERATE	*Level 2 practices plus*: special protective clothing, controlled access through entrance room, biological waste must be autoclaved; preferably in facility.	Partial containment equipment used for all manipulations of infectious materials, directional airflow.	Yellow fever, *M. tuberculosis*, Short term culture of tissue from non-human primates until cultures are known to be free of Herpes-virus *simiae* (B. virus)
BL4 **Maximum Containment Laboratory**	Individual risk: HIGH Community risk: HIGH	*Level 3 practices plus:* entrance through change room. Complete change of clothing from street to laboratory gear, shower at exit. All wastes decontaminated on exit from facility.	Maximum containment equipment (i.e., Class III Biosafety cabinet or partial containment in combination with full-body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	Ebola-Marburg Virus. Propagation of Herpes virus *simiae.*

SUMMARY OF ANIMAL FACILITY BIOSAFETY LEVELS

BL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
BL1-N	Not known to consistently cause disease in healthy human adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species.	Standard animal facility. No recirculation of exhaust air. Directional air flow recommended Handwashing sink recommended
BL2-N	Associated with human disease. Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	BL1-N practices plus: Limited access. Biohazard warning signs. Sharps precautions. Biosafety manual. Decontamination of all infectious wastes and of animal cages prior to washing	BL1-N equipment plus primary barriers: containment equipment appropriate for animal species; PPEs: laboratory coats, gloves, face and respiratory protection as needed.	BL1-N facility plus: Autoclave available. Handwashing sink available in the animal room. Mechanical cage washer used.
BL3-N	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects.	BL2-N practices plus: Controlled access. Decontamination of clothing before laundering. Cages decontaminated before bedding removed. Disinfectant foot bath as needed	BL2-N equipment plus: Containment equipment for housing animals and cage dumping activities. Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: appropriate respiratory protection	BL2-N facility plus: Physical separation from access corridors. Self-closing, double-door access. Sealed penetrations. Sealed windows. Autoclave available in facility
BL4-N	Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown risk of transmission	BL3-N practices plus: Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting. All wastes are decontaminated before removal from the facility	BL3-N equipment plus: Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities	BL3-N facility plus: Separate building or isolated zone. Dedicated supply and exhaust, vacuum and decontamination systems. Other requirements outlined in BMBL-4