NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Department of Environment, Health and Safety - 04/2013

This is the print version of the Recombinant or Synthetic Nucleic Acid Molecules Training Module. It does not contain the Knowledge Review, or the test questions. If you have questions, please contact the Department of Environment, Health and Safety at 919-962-5722.

Course Overview

Goals of the Training

Upon completion of this training, the Principal Investigator should understand: General requirements under the:

- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules Research.
- His/her responsibilities under the NIH Guidelines.

Principal Investigators and Other Researchers:

The course provides information in order for the principal investigator to make sure that his/her laboratory complies with the National Institute of Health Guidelines for such research.

In addition, the Guidelines identify key ethical principles and key safety reporting requirements, applicable to all researchers who work with recombinant or synthetic nucleic acid molecules.

Training Requirements

A Knowledge Review will follow each module in order to provide a learning experience for that module. You may skip the learning modules if you choose.

If you do not work with recombinant or synthetic nucleic acid molecules or transgenic animals/plants, the training program will end with a test at the end of Module Two. You may retake the test at any time.

If you work with recombinant or synthetic nucleic acid molecules or transgenic animals/plants, you must complete Modules 1, 2, 3, 4, 5, and 6 to determine what category your research falls under, and if IBC approval is required. The training will end with a test at the end of the last module: Module Six. You may retake the test at any time.

Learning Aids

To aid you in your understanding of the NIH Guidelines, you can also find the following documents at any time throughout this module by clicking on the links at the top of each frame.

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
NIH Office of Biotechnology Website
Biosafety Considerations for Research with Lentiviral Vectors
NIH Report - Assessment of Adenoviral Vector Safety and Toxicity (pdf)

Program Modules

There are six training modules:

Module One: Introduction to the NIH Guidelines and Scope
Module Two: Roles and Responsibilities
Module Three: Types of Experiments Covered
Module Four: Safety Considerations or Risk Assessment
Module Five: Submission to the IBC and IBC Review
Module Six: Incident Reporting

Introduction to the NIH Guidelines

NIH Office of Biotechnology Activities

The NIH Office:

1. Oversees recombinant or synthetic nucleic acid molecules research, including human gene transfer.
2. Manages the Recombinant DNA Advisory Committee (RAC), a public advisory committee that advises the Department of Health and Human Services and NIH about recombinant or synthetic nucleic acid molecule research.
3. Administers the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

4. Partners with Institutional Biosafety Committees in the oversight of recombinant DNA research.

History of the Guidelines Development

With the emergence of recombinant DNA technology in the mid- 1970s, the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules grew out of concerns by scientists and the general public for public health and safety, environmental impact, and the ethical and social implications of such research.

The original guidelines were issued in 1976. Multiple revisions have been issued since, with the last revisions issued in March 2013.

Scope of the Guidelines

The purpose of the NIH Guidelines is to specify practices for constructing and handling:

1. Recombinant DNA (rDNA) molecules
2. Nucleic acid molecules produced solely by synthetic means
3. Organisms and viruses containing rDNA molecules
4. Some transgenic animals

Recombinant DNA Molecules

Definition:

- Molecules that are constructed outside living cells by joining natural or synthetic DNA to DNA molecules that can replicate in a living cell
- Molecules that result from the replication of the molecules described above
- Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (i.e. toxin) are exempt unless they are expressed in vivo as a biologically active product.
- Genomic DNA of plants and bacteria that have acquired a transposable element (even if it was donated from a recombinant vector that is no longer present) are exempt unless the transposon itself contains rDNA.

SiRNA

Definition:

- Small interfering RNAs are a class of 20-25 nucleotide-long double-stranded RNA molecules involved in the RNA interference (RNAi) pathway and interferes with the expression of a specific gene.

- Recombinant DNA experiments include inserting DNA encoding siRNA targeting the gene of interest into an expression vector

Synthetic Nucleic Acids

Definition:

- 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 acids, i.e. synthetic NA
- Synthetic techniques may enable the synthesis of more complex chimeras containing sequences from a number of different sources.

Transgenic Animals

Definition:

- Animals in which there has been a deliberate modification of the genome in contrast to spontaneous mutation.
- Foreign DNA is introduced into the animal using recombinant or synthetic nucleic acid molecule technology, and then must be transmitted through the germ line so that every cell, including germ cells of the animal, contain the same modified genetic material.

Cre/loxP System

Example:

mice can be made transgenic for the gene encoding Cre attached to a promoter activated only when bound by the appropriate transcription factors:

1. A "target" gene is flanked by loxP sequences.
2. Transcription of the Cre gene. Its protein will then remove the "target" gene under study.
3. The result: a mouse with a particular gene knocked out in only certain cells.

NIH Guidelines are Applicable to:

- recombinant or synthetic nucleic acid research conducted at or sponsored by an institution that receives any support for recombinant or synthetic nucleic acid research from the NIH;
- Research that involves testing in humans of materials containing recombinant or synthetic nucleic acid molecules.

UNC-CH receives NIH support. Therefore, all recombinant or synthetic nucleic acid

molecule work conducted at UNC must be in compliance. All research with eligible synthetic nucleic acid molecules must be registered by March 5, 2013.

Non-Compliance Consequences

Non-compliance with the NIH Guidelines can result in:

1. Suspension, limitation or termination of NIH funding for UNC-Chapel Hill.
2. A requirement of prior NIH approval of all recombinant or synthetic nucleic acid molecule projects at UNC.

Roles and Responsibilities

The NIH Guidelines will never be complete or final since all conceivable experiments involving recombinant or synthetic nucleic acid molecules 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.
-Section IV-A, Roles and Responsibilities: Policy, NIH Guidelines

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Roles and Responsibilities of:

- Institution (UNC-Chapel Hill)
- Institutional Biosafety Committee (IBC)
- Environment, Health and Safety (EHS)
- Principal Investigator (PI)
- NIH

Institutional (UNC's) Responsibilities

UNC-CH is responsible for:

1. Establishing and implementing policies for the safe conduct of recombinant or synthetic nucleic acid molecules research;
2. Establishing an Institutional Biosafety Committee;
3. Assisting and ensuring compliance with the NIH Guidelines by investigators;
4. Ensuring appropriate training for IBC members and staff, PIs and laboratory staff;
5. Reporting any significant problems or violations to OBA.

Institutional Biosafety Committee (IBC) Responsibilities

The IBC is responsible for:

1. Reviewing, approving and overseeing recombinant or synthetic nucleic acid molecules research conducted at UNC to ensure compliance with the Guidelines;

2. Periodically reviewing recombinant or synthetic nucleic acid moleculesresearch;
3. Setting containment levels;
4. Reporting significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses to the NIH/OBA.

Environment, Health and Safety Responsibilities

The Dept. of Environment, Health and Safety is responsible for:

1. Conducting periodic inspections of laboratories working with recombinant or synthetic nucleic acid molecules.
2. Reporting any significant problems or violations of the NIH Guidelines or any significant research-related accidents or illnesses to the IBC.
3. Developing emergency plans for spills.
4. Investigating laboratory accidents.
5. Providing advice on lab security.
6. Providing technical advice to PIs and IBC

Principal Investigator Responsibilities

1. PI is responsible for full compliance with the NIH Guidelines.
2. PI must determine if experiments require IBC approval.
3. Experiments that require prior IBC approval cannot be conducted until IBC approval is obtained.
4. Experiments that require registration (Section III-E) must not begin until the appropriate paperwork has been submitted to the IBC.
5. Make initial determination of containment levels required for experiments. (See Risk Assessment Module Four)
6. Notify the IBC of any changes to recombinant or synthetic nucleic acid molecule experiments previously approved by the IBC.
7. Immediately report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to Environment, Health and Safety (962-5507) and the IBC.
8. Have an updated Laboratory Safety Plan on file at EHS and in their Laboratory Safety Notebook. Describe practices required to ensure safety on Schedule F and annually train laboratory staff.
9. Determine appropriate medical surveillance required for work with recombinant or synthetic nucleic acid molecules. This must be described on Schedule F.

10. The PI is required annually to respond to an IBC request of whether or not their protocols are active.

Principal Investigator's Responsibilities for Human Use Protocols

1. Principal investigators must report any serious adverse events associated with the use of rDNA or synthetic nucleic acid molecules in humans to the IBC.
2. A serious adverse event is an event occurring at any dose that results in any of the following outcomes: death, life threatening event, in-patient hospitalization, prolongation of existing hospitalization, persistent or significant disability/incapacity, or congenital anomaly/birth defect.
3. An adverse event is associated with the use of a gene transfer product when there is a reasonable possibility that the event may have been caused by the use of that product.
4. If synthetic nucleic acid molecules, or DNA or RNA derived from them will be administered to human subjects, the research must be registered if **any** of the following conditions are met:
    a. Molecules contain more than 100 nucleotides.
    b. Molecules can integrate into the genome.
    c. Molecules have the potential to replicate in a cell.
    d. Molecules can be translated or transcribed.

5. Bench research with synthetic nucleic acid molecules must be registered if:
    a. The molecules can replicate.
    b. The molecules can generate nucleic acids that can replicate in a living cell.
    c. The molecules can integrate into a host cell's DNA.
    d. They can produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms/kilogram of body weight.
    e. They can synthesize an organism that doesn't naturally occur outside of a laboratory setting (i.e. 1918 H1N1 influenza, wild-type polio virus).

Module Two: NIH Responsibilities

1. Managing the RAC.
2. Conducting and supporting training of IBCs.
3. Review of human gene transfer protocols.
4. Review of certain basic recombinant DNA or synthetic nucleic acids experiments:
5. Deliberate transfer of drug resistance trait to microorganisms not known to acquire the trait naturally, if it could compromise disease control
6. Cloning of toxin molecules with LD50<100 ng/Kg bodyweight

7. DNA from restricted agents transferred to nonpathogenic prokaryotes or lower eukaryotes
8. DNA from nonpathogenic prokaryotes or lower eukaryotes transferred to restricted agents
9. Use of infectious or defective restricted poxviruses in presence of helper virus

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Please review the following instructions before proceeding to the next Module.

If you do not work with recombinant DNA, synthetic nucleic acids or transgenic animals/plants, this completes the information that you will be tested upon.

If, however, you work with recombinant DNA, synthetic nucleic acids or transgenic animals or plants, you must complete Modules 3, 4, 5 and 6 to determine what category your research falls under, and if IBC approval is required.

Experiments Covered

Experiments Covered by the NIH Guidelines

Experiment Types	Review Required	Section
Transfer of drug resistance traits that would affect control of disease.	IBC, RAC, NIH/OBA prior to initiation of experiments	III-A
rDNA or synthetic nucleic acids containing genes for synthesis of toxins with LD50 < 100 ng/kg	IBC, NIH/OBA prior to initiation of experiments	III-B
Transfer of rDNA or synthetic nucleic acids into human subjects	IBC, RAC, IRB prior to initiation of experiments	III-C
rDNA or synthetic nucleic acids from Risk Group 2, 3, 4 or restricted agents or use as host vector systems. Some experiments involving whole animals or plants. Large scale experiments.	IBC prior to initiation of experiments	III-D (Examples: adenoviral vectors, lentiviral vectors, retroviral vectors, VRP vectors)
Formation of rDNA or synthetic nucleic acids comprised of < 2/3 eukaryotic virus genome (proposed to change to ½ of the genome). Some experiments with whole plants. Creation of transgenic rodents that require BSL1 containment.	IBC notice simultaneous with initiation of experiments.	III-E
rDNA or synthetic nucleic acids not in	Exempt from NIH	III-F

organisms or viruses. Purchase or transfer of transgenic rodents that require BSL1 containment.	Guidelines. IBC approval required to assess biosafety levels and containment.	Exempt from NIH Guidelines but UNC IBC approval is required to assess biosafety containment of the animals.

Next Step for the PI

Once the PI has determined which category of the NIH Guidelines their research falls under, they must then conduct a risk assessment to determine the practices and containment that must be used when conducting their experiments.

Risk Assessment

Principal Investigator's Risk Assessment

Principal Investigator must conduct a comprehensive risk assessment in order to:

1. Determine risk group of the agent - Appendix B
2. Evaluate agent factors
    a. Virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, quantity, availability of vaccine or treatment
        i. Strain that is known to be more hazardous than wild type should be considered for handling at a higher containment level.
        ii. Attenuated strains may be able to be handled at lower containment.
3. Evaluate gene product effects:
    a. toxicity, physiological activity and allergenicity
4. Evaluate how the agent will be used:
    a. Animal experiments may require higher containment, or large quantity production (>10 L) may require higher containment.
5. Determine appropriate laboratory containment and practices required based on the above information.
    a. Containment levels are described in Appendix G

Risk Groups

There are four risk groups:

Risk Group 1	Risk Group 2	Risk Group 3	Risk Group 4
Agents are not associated with disease in healthy adult humans.	Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.	Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.	Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are usually not available.

Risk Group 1

Agents are not associated with disease in healthy adult humans.

Examples

1. E. coli K-12
   That does not possess a complete lipopolysaccharide
   That does not carry any active virulence or colonization factors or genes encoding these factors
2. Bacillus subtilis host vector systems
3. Adeno-associated virus (AAV) types 1-4
4. Recombinant AAV constructs produced in the absence of helper virus
   transgene cannot encode for tumorigenic gene product or toxin molecule

Risk Group 1, con't

Animal Viral Etiologic Agents in Common Use
Not associated with disease in healthy human adults

Examples

1. Murine Cytomegalovirus
2. Bovine Papilloma virus
3. Feline leukemia virus
4. Murine leukemia virus (exception: amphotropic and xenotropic strains are infectious to humans and are considered Risk Group 2).

See Appendix B-V

Risk Group 2

Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.

Examples

1. *Staphylococcus aureus*
2. *Streptococcus*
3. *Cryptococcus neoformans*
4. *Giardia* sp.
5. Human adenoviruses
6. Herpesviruses (expect Monkey B)
7. Influenza viruses

See Appendix B-II

Risk Group 3

Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.

Examples

1. *Mycobacterium tuberculosis*
2. Human immunodeficiency virus (HIV) types 1 and 2
3. Venezuelan equine encephalomyelitis (VEE) virus

See Appendix B-III

Risk Group 4

Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are usually not available.

Examples

1. Ebola virus
2. Herpes B virus

See Appendix B-IV

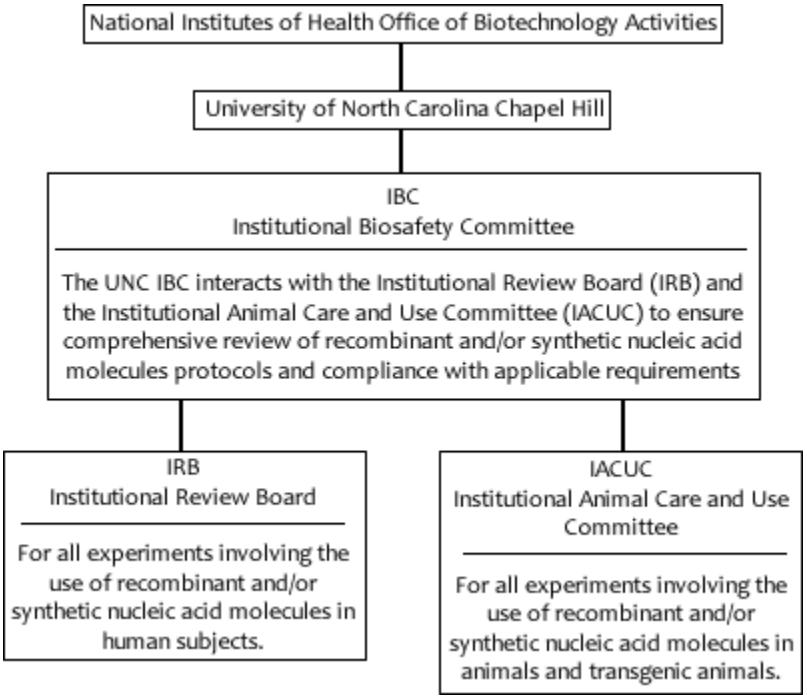
Submission to IBC and IBC Review

Submission of rDNA Registrations to IBC

NIH Sections and/or Experiments	Required Schedule or Appendix
III-A, III-B, III-D, III-E	G
Purchase or transfer of transgenic	H
III-C (Human Use)	1. Appendix 10A, plus, 2. Scientific abstract, 3. non-technical abstract, 4. Responses to Appendix M-II through M-V, 5. clinical protocol, 6. informed consent document, 7. curriculum vitae--no more than two pages for each key professional person in biographical sketch format.

Oversight

The UNC IBC interacts with other committees to ensure comprehensive review of rDNA protocols and compliance with applicable requirements.



Animal Use Experiments

Experiments involving the use of animals also require UNC IACUC approval before they can be initiated. IACUC applications can be initiated at https://cfx2.research.unc.edu/acap/.

IBC and IACUC Review of Animal Research Utilizing Recombinant or Synthetic Nucleic Acid Molecules

IBC Review

1. Risks to human health while working with rDNA or synthetic nucleic acid vectors.
2. Risks to the environment including escape of the vector or animal and establishment in the wild and altered animals interbreeding with wild stock
3. IBC approval is required for any animal experiments that involve rDNA, synthetic nucleic acids or any transgenic animals before the IACUC protocol is approved.

IACUC Review

Animal welfare concerns including:

1. Pain and distress from adverse phenotypes (behavioral, anatomical and physiological abnormalities)
2. Risks to other animals in the facility from the inadvertent spread of vectors

See: IACUC webpage

Human Use Experiments

Human use experiments also require UNC IRB approval before they can be initiated. IRB submission information can be found at:

Research At Carolina

Office of Human Research Ethics - Guide to the IRB Process

IBC and IRB Review of Research with Human Subjects

IRB Review

1. Conducts risk/benefit assessment relative to individual research participants (physical, psychological, social harms)
2. Selection of subjects and the informed consent process
3. Data monitoring provisions to ensure the safety of subjects

4. Provisions to protect subject privacy and confidentiality of data
5. Injuries or any other unanticipated problems
6. Compliance with regulations

See: A guide to the IRB process

IBC Review

1. Recombinant or synthetic nucleic acid molecules research for conformity with the NIH Guidelines
2. Potential risk to environment and public health, community, as well as to individual research participants
3. Containment levels per NIH Guidelines
4. Adequacy of facilities, SOPs, PI and other personnel training
5. Institutional and investigator compliance (e.g., adverse event reports)
6. Reviews trial design, biosafety and containment, and compliance with NIH Guidelines

IBC approval is required for any human use experiments that involve recombinant or synthetic nucleic acid molecules before the IRB protocol is approved.

Procedures

Submission of rDNA and/or synthetic nucleic acid Registrations to IBC

1. Forms in the online lab safety plan must be filled out completely and approved online by the Principal Investigator.
2. The Principal Investigator signing the form must have a current Laboratory Safety Plan on file with EHS.

Not sure if your plan is current? Please call EHS at 919-962-5507

All Schedule G or Schedule H forms will be submitted online via the online lab safety plan.

3. IBC meets the first Monday of each month. Forms should be submitted by the 15th of the month prior to being reviewed at the meeting.
4. If registration is approved, the form is signed by the IBC chair and a copy is provided to the Principal Investigator via email.
5. A copy of the approval must be maintained in the Principal Investigator's Laboratory Safety Notebook for documentation that experiments are approved.

Incident Reporting

Requirements for Reporting

NIH Guidelines require reporting of the following to the OBA:

1. Significant problems
2. Violations of the NIH Guidelines
3. Research-related accidents and illnesses

UNC requires reporting of ALL lab related incidents to the Department of Environment, Health and Safety (962-5507) on the same business day if the incident occurs during normal work hours and on the next business day if the incident occurs after normal work hours.

Incident Definition

1. Needlesticks
2. Animal bites
3. Released or lost transgenic or infected animals
4. Uncontained spills
5. Punctured or breached PPE
6. Splashes to eyes/mucous membranes
7. Accidental aerosolization/inhalation
8. Inappropriate waste disposal
9. Illness/symptoms related to agents in lab
10. Any other incident in which researcher is unsure about exposure potential.

Or, any event that leads to unintended exposure.

Incident Procedures

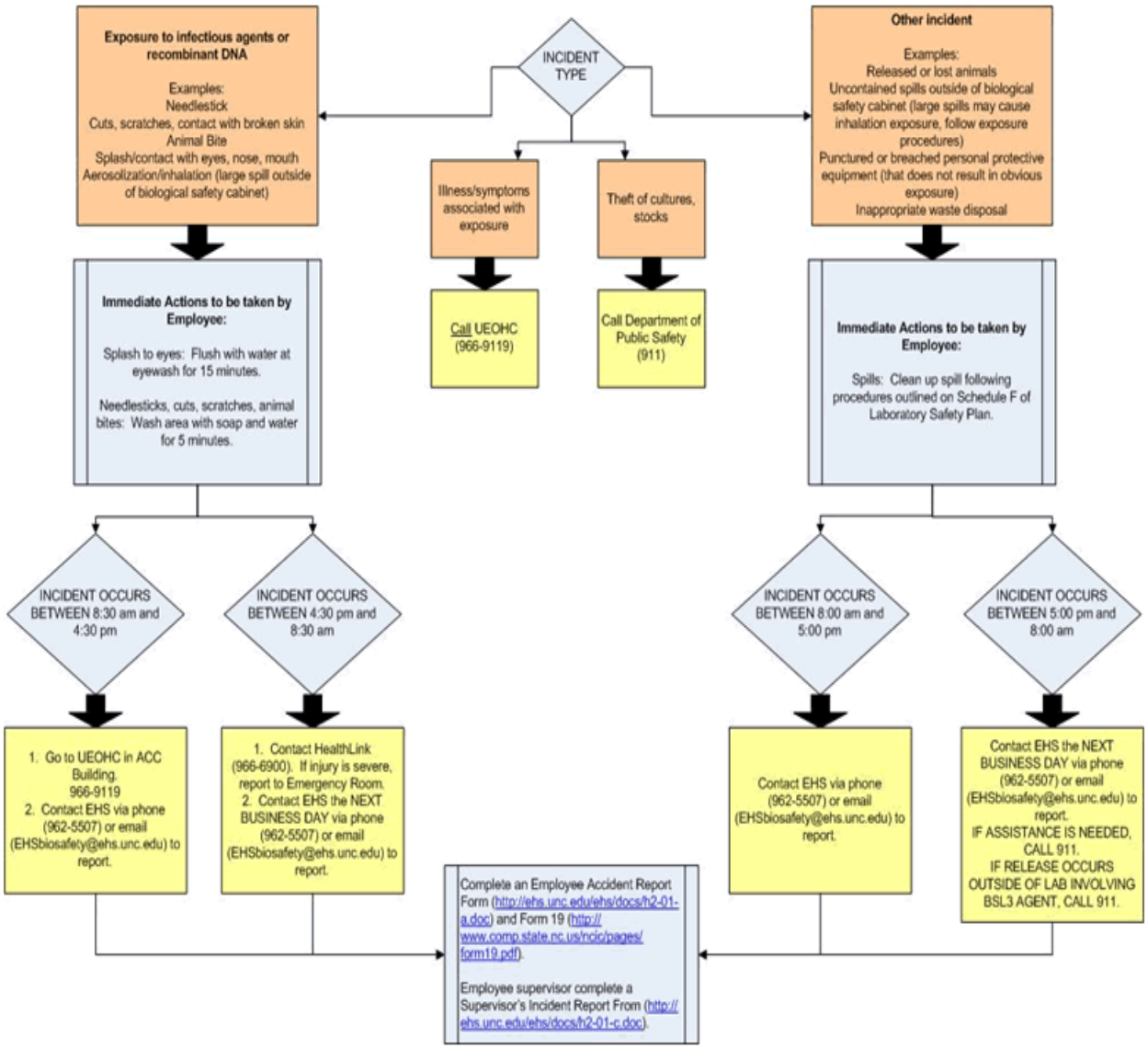
PIs are required to describe procedures for spills and exposures on Schedule F of their Laboratory Safety Plan.

How to Report Incidents

Following the Initial Report of the Incident

1. Employees will be required to fill out an Employee Accident Report Form
2. Supervisors are required to fill out a Supervisors Incident Report Form
3. The Employee will also have to fill out a Form 19.

1. These procedures must include what steps to take after-hours (i.e. go to Emergency Room or report next business day)



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If you have questions about this training program, please contact Deborah Howard at 962-5722.