

# Haynes Lab SOP

## Standard Operating Procedures

PI: Karmella A. Haynes

Location: **ISTB-4, 231** (open lab)  
231A (tissue culture)  
231B (microscopy)  
LER (freezers, gas cylinders)

Last Updated: February 2014

### Current Procedures For:

- Hazardous chemicals
- Human cell culture
- Microbes (E. coli, Yeast)
- Recombinant DNA
- Gas cylinders

### **SAFETY TRAINING**

The following training courses are required before working in the Haynes Lab:

1. Laboratory Safety
2. Fire Safety & Prevention
3. Gas Cylinders (all personnel except undergraduate volunteers)
4. Biosafety and Bloodborne Pathogens
5. Hazardous Waste Management
6. NIH Recombinant DNA Training

Instructions for signing up for these courses are at  
<http://openwetware.org/wiki/Haynes:Internal>

## **SAFETY RULES**

### ***Required Personal Protective Equipment***

Close-toed shoes

Lab coat

Disposable gloves

## **CHEMICAL INVENTORY**

To be updated once a year



# Biosafety Manual

Arizona State University  
Department of Environmental Health & Safety

Last Updated: **July 2012**

## FOREWORD

The Biosafety Manual has been adopted by Arizona State University to be a resource for information, guidelines, policies, and procedures that will enable and encourage those working in the laboratory environment to work safely and to eliminate, or reduce, the potential for exposure to biological hazards. The Department of Environmental Health and Safety has developed this manual to help ensure compliance with the following federal, state, and local regulations:

- 7 Code of Federal Regulations § 331
- 9 Code of Federal Regulations § 121
- 18 United States Code § 175b
- 29 Code of Federal Regulations § 1910.1030
- 42 Code of Federal Regulations § 72
- 42 Code of Federal Regulations § 73
- 42 Code of Federal Regulations § 1003
- 49 Code of Federal Regulations, Parts 171-180
- Arizona Administrative Code, Article 14, Biohazardous Medical Waste and Discarded Drugs
- Centers for Disease Control & Prevention/National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*.
- National Institutes of Health. *Guidelines for Research Involving Recombinant DNA Molecules*.
- Public Act 107-188, HR3448

The Biosafety Manual provides a compilation of suggested work practices, protocols and systems to work safely at Arizona State University. The Biosafety Manual should not be considered the only reference for health and safety concerns. It is intended that the Principal Investigator (PI) and supervisory personnel will supplement this information with instruction and guidance regarding specific practices and procedures unique to the work being done in their laboratories. In addition, the Department of Environmental Health and Safety is always available to address health and safety concerns.

The Biosafety Manual will be reviewed at least annually by the Department of Environmental Health and Safety and the Institutional Biosafety Committee.



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Leon Igras, Director  
Environmental Health and Safety

\_\_\_\_\_  
July 19, 2012

Date



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David Gillum, Biosafety Officer  
Environmental Health and Safety

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July 19, 2012

Date

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## I. PREFACE

The Arizona State University (ASU) Biosafety Manual is intended to be a resource for information, guidelines, policies, and procedures that will enable and encourage those working in the laboratory environment to work safely and to eliminate, or reduce, the potential for exposure to biological hazards. The information presented here also reflects the requirements and guidelines of federal and state regulations. The most current version of the ASU Biosafety Manual is available on-line on the ASU [Environmental Health & Safety](#) (EH&S) website.

## II. INTRODUCTION

### A. **Scope**

The ASU Biosafety Manual is applicable to all laboratory, research, teaching, service, and support activities that may involve exposure to biohazards. Biohazards are microorganisms, microbial toxins, or other biological agents that can infect and/or cause disease in humans, animals, or plants. Biohazards may include bacteria, bacterial toxins, viruses, fungi, rickettsia, prions, protozoans, parasites, genetically-modified organisms, or recombinant DNA molecules. In addition, biohazards include human blood, body fluid, tissues, and cell lines of human origin. Biohazards are often referred to as infectious agents or etiological agents.

Biosafety encompasses the knowledge, techniques, equipment, and facilities necessary to prevent or minimize an exposure to, or release of, a biological agent. The mission of the Biosafety Division in the ASU Department of Environmental Health and Safety is to assure a safe and healthy environment for individuals working with biohazards and to protect the community and environment by preventing the release and exposure to biohazards.

### B. **Regulatory Requirements and Guidelines**

Guidance documents from the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) form the basis for the biosafety practices included in this manual. There are additional guidance documents and regulations imposed by various funding agencies that individual PIs must be aware of and incorporate into their **Lab-Specific Biosafety Manuals**. Biosafety requirements must be followed to ensure the continuation of grant funding from federal agencies and for health and safety purposes.

The NIH [Guidelines for Research Involving Recombinant DNA Molecules](#) (*NIH Guidelines*) detail procedures and practices for the containment and safe conduct of various forms of recombinant DNA research, including research involving genetically modified plants and animals, as well as human gene transfer. The *NIH Guidelines*:

- Mandate the establishment of an Institutional Biosafety Committee for the review and oversight of biological research;
- Outline roles and responsibilities for biosafety; and
- Establish the practices, procedures, and conditions under which recombinant DNA activities must be conducted.

All institutions, including ASU, receiving NIH funding for recombinant DNA activities must comply with the *NIH Guidelines*. Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if their individual projects are not funded by NIH. **Non-compliance with the *NIH Guidelines* may result in suspension, limitation, or termination of financial assistance** for the research project and of NIH funds for other recombinant DNA activities at ASU or the requirement for prior NIH approval of any and/or all recombinant DNA projects at ASU.



The CDC/NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL), describes the appropriate measures and facilities for work with all microbial agents, including bacterial, viral, fungal, parasitic, rickettsial, and prion agents as well as toxins of biological origin.

The requirements described in the Occupational Safety and Health Administration’s (OSHA) [Bloodborne Pathogens](#) regulation (29 CFR § 1910.1030) apply to work with human blood, tissue, organs, body fluids, and cell cultures. Special training, medical surveillance, procedures, and equipment that must be in place for protection against bloodborne pathogens, needlesticks, and other sharps injuries, are described in the [ASU Exposure Control Plan](#).

Handling and disposal of biohazardous waste is regulated by OSHA under the [Bloodborne Pathogens](#) regulation and by state and federal statutes. The procedures for biohazardous waste handling are described in the [ASU Biological Waste Handling Procedures](#).

The requirements for packaging and shipment of biohazardous materials are provided in the Department of Transportation’s hazardous materials regulation [49 CFR, Parts 171 – 180](#). In addition, permits may be required to ship biological materials. Please refer to the CDC [Etiological Agent Import Permit Program](#) and the Animal and Plant Health Inspection Service (APHIS) [permit program](#). Information on shipping procedures that comply with these regulations is found in the section on “Shipping and Transportation Methods and Requirements” in this manual.

Specific requirements for handling toxins found in 29 CFR § 1910.1450 [Occupational Exposure to Hazardous Chemicals in Laboratories](#) are covered in the ASU [Chemical Hygiene Plan](#). Information on ASU’s radiation safety program is found in the [Radioactive Materials Manual](#).

### C. Select Agents and Toxins

Select agents are particular microorganisms and toxins specifically identified in federal regulations. Select agents also include specific genetically modified microorganisms or genetic elements that contain specific nucleic acid sequences. The most current list is found on the CDC’s website at <http://www.selectagents.gov/>.

The following toxins are not regulated as select agents or toxins if the amount under the control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the table below.

HHS Select Agent Toxins	CAS #	Threshold Amount
Abrin	1393-62-0	100 mg
Botulinum neurotoxins	93384-43-1	0.5 mg
Clostridium perfringens epsilon toxin		100 mg
Conotoxin	76862-65-2 / 156467-85-5	100 mg
Diacetoxyscirpenol (DAS)	2270-40-8	1000 mg
Ricin	96638-28-7	100 mg
Saxitoxin	35523-89-8	100 mg
Shiga-like ribosome inactivating proteins		100 mg
Shigatoxin	75757-64-1	100 mg
Staphylococcal enterotoxins	11100-45-1	5 mg
T-2 toxin	21259-20-1	1000 mg
Tetrodotoxin	4368-28-9	100 mg

The ASU Biosafety Manual, when used in combination with the **Lab-Specific Biosafety Manual**, is designed to meet the federal requirements of the Department of Health and Human Services (HHS) Standard "[Possession, Use, and Transfer of Select Agents and Toxins; Final Rule](#)" (42 CFR § 73) and the Department of Agriculture (USDA) Standard, "[Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins; Final Rule](#)" (7 CFR § 331 and 9 CFR § 121). All laboratory specific requirements for these regulations can be found in the appendix titled "Special Considerations for Select Agents and Toxins."

#### D. Risk Group Classifications

According to the CDC/NIH document, [Biosafety in Microbiological and Biomedical Laboratories](#), also known as the BMBL, the three primary hazardous characteristics associated with biological agents include:

- The capability of an agent to infect and cause disease in a susceptible human or animal host;
- The virulence of an agent as measured by the severity of disease; and
- The availability of preventive measures and effective treatments for the disease.

By taking the route of transmission of the disease into consideration, a standardized methodology was developed to classify biological agents into four different risk groups (please see the table below). Knowing the risk group of an agent assists researchers and safety professionals in determining the appropriate safety protocols to be followed.

Risk Group (RG) Classifications			
RG-1	RG-2	RG-3	RG-4
Agents that are not associated with disease in healthy adult humans.	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

#### E. Laboratory Biosafety Levels

CDC and NIH have established four levels of biosafety, based on the degree of hazard associated with a microbial agent, to describe the combination of laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure. The BMBL outlines four different biological safety levels that are appropriate for the operations performed in a laboratory, the documented or suspected routes of transmission of the biological agent, and the laboratory function or activity. These four biosafety levels (BSL) require successively more stringent practices and facilities as work moves from the least restrictive, BSL-1, to work with the highest hazard level of BSL-4. Exposure to biohazardous agents may be prevented or limited by establishing and following the appropriate biosafety level practices and conditions. The requirements for each laboratory biosafety level can be found in the [BMBL](#).

The following bullets provide a brief summary of the four biological safety levels:

- **BSL-1** is required for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment.

- **BSL-2** is required for work involving agents that pose moderate hazards to personnel and the environment.
- **BSL-3** is required for clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.
- **BSL-4** is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.

**Note:** No research with biohazardous agents at BSL-4 is permitted in Arizona State University facilities.

Personal protective equipment varies depending upon the biological safety level. Please refer to the table below for specific requirements for each of the four biological safety levels.

<b>Biological Safety - Personal Protective Equipment (PPE) Requirements*</b>			
<b>BSL-1</b>	<b>BSL-2</b>	<b>BSL-3</b>	<b>BSL-4</b>
<ul style="list-style-type: none"> <li>• <b>Protective laboratory coats, gowns, or uniforms</b> recommended preventing contamination of personal clothing.</li> <li>• <b>Protective eyewear</b> worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.</li> <li>• Personnel who wear contact lenses in laboratories should also wear eye protection.</li> <li>• <b>Gloves</b> must be worn to protect hands from exposure to hazardous materials.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Protective laboratory coats, gowns, smocks, or uniforms</b> must be worn while working with hazardous materials.</li> <li>• <b>Eye and face protection</b> (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms are handled outside the Biological Safety Cabinet (BSC) or physical containment device.</li> <li>• Personnel who wear contact lenses in laboratories should also wear eye protection.</li> <li>• <b>Gloves</b> must be worn to protect hands from exposure to hazardous materials.</li> <li>• <b>Eye, face and respiratory protection</b> should be used in rooms containing infected animals.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Protective laboratory clothing with a solid-front</b>, such as tie-back or wrap-around gowns, scrub suits, or coveralls must be worn.</li> <li>• <b>Eye and face protection</b> (goggles, mask, face shield or other splash guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials. [All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices.]</li> <li>• Personnel who wear contact lenses in laboratories must also wear eye protection.</li> <li>• <b>Gloves</b> must be worn to protect hands from exposure to hazardous materials.</li> <li>• <b>Eye, face, and respiratory protection</b> must be used in rooms containing infected animals.</li> </ul>	<ul style="list-style-type: none"> <li>• Not permitted at ASU.</li> <li>• Please refer to the CDC/NIH document, "Biosafety in Microbiological and Biomedical Laboratories" for PPE requirements.</li> </ul>

\* Safety is improved when PPE is used in combination with physical containment devices or equipment, such as Biological Safety Cabinets (BSCs).

## F. Animal Biosafety Levels

Similar to the BSL, there are four animal biosafety levels (ABSL). These four animal biosafety levels are required for the use of experimentally-infected animals housed in indoor research facilities (e.g., vivaria), and also in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. **As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents in vivo and in vitro are comparable with the animal biosafety level.**

The four animal biosafety levels provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to Biosafety Levels 1-4, respectively. **Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.**

The following bullets provide a brief summary of the four biological safety levels:

- **ABSL-1** is required for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.
- **ABSL-2** is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment, and also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.
- **ABSL-3** is required for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease.
- **ABSL-4** is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments; or a related agent with unknown risk of transmission.

**Note:** No research with biohazardous agents at BSL-4 is permitted in Arizona State University facilities.

In addition to animal biosafety consideration, laboratory animal facilities, operational practices, and quality of animal care must meet applicable standards and regulations (e.g., *Guide for the Care and Use of Laboratory Animals* and *Laboratory Animal Welfare Regulations*) and that appropriate species have been selected for animal experiments. The USDA has also developed facility parameters and work practices for handling agents of agriculture significance.

Personal protective equipment varies depending upon the biological safety level. Please refer to the following table for specific requirements for each of the four biological safety levels.

<b>Animal Biological Safety - Personal Protective Equipment (PPE) Requirements*</b>			
<b>ABSL-1</b>	<b>ABSL-2</b>	<b>ABSL-3</b>	<b>ABSL-4</b>
<ul style="list-style-type: none"> <li>• <b>Protective laboratory coats, gowns, or uniforms</b> recommended to prevent contamination of personal clothing.</li> <li>• <b>Eye, face, and respiratory protection</b> should be used in rooms containing infected animals.</li> <li>• <b>Protective eyewear</b> must be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.</li> <li>• Personnel who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.</li> <li>• <b>Gloves</b> must be worn to prevent skin contact with contaminated, infectious, and hazardous materials, and when handling animals.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Protective laboratory coats, gowns, or uniforms</b> must be worn while in areas where infectious materials and/or animals are housed or manipulated.</li> <li>• <b>Eye and face protection</b> (mask, goggles, face shield or other splatter guard) must be worn when performing manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or physical containment device.</li> <li>• Personnel who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.</li> <li>• <b>Gloves</b> must be worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.</li> <li>• <b>Eye, face, and respiratory protection</b> should be used in rooms containing infected animals.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Disposable personal protective equipment, such as non-woven olefin cover-all suits, wrap-around or solid-front gowns</b>, should be worn (over uniforms or scrub suits) before entering areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.</li> <li>• <b>Eye, face, and respiratory protection</b> must be used in rooms containing infectious materials and in areas where animals are housed or manipulated. [All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices.]</li> <li>• Personnel who wear contact lenses in laboratories must also wear eye protection.</li> <li>• <b>Gloves</b> must be worn to prevent skin contact with contaminated, infectious, and hazardous materials and when handling animals. Double-glove practices should be used.</li> <li>• <b>Boots, shoe covers, or other protective footwear</b>, are used to prevent cross-contamination.</li> </ul>	<ul style="list-style-type: none"> <li>• Not permitted at ASU.</li> <li>• Please refer to the CDC/NIH document, "Biosafety in Microbiological and Biomedical Laboratories" for PPE requirements.</li> </ul>

\* Safety is improved when PPE is used in combination with physical containment devices or equipment, such as Biological Safety Cabinets (BSCs).

It is the responsibility of institutional management to provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for the laboratory animal. There are unique hazards associated with infected animals that must be understood by personnel with animal contact. Animal activity may create aerosols, and bites and scratches can occur.

## **G. Biohazardous Waste**

According to the State of Arizona, biohazardous waste is defined as:

- 1) Any solid waste which is generated in the diagnosis, treatment, or immunization of a human being or animal or in any research relating to that diagnosis, treatment, or immunization, or in the production or testing of biological materials.
- 2) Animal carcasses, body parts, and bedding of animals that have been infected with agents that produce, or may produce, human infection.
- 3) Discarded cultures and stocks generated in the diagnosis, treatment, or immunization of a human being or animal or in any research relating to that diagnosis, treatment or immunization, or in the production or testing of biological.
- 4) Discarded products and materials containing blood or blood components.
- 5) Discarded organs and body parts removed during surgery.
- 6) Discarded sharps used in animal or human patient care, medical research, or clinical laboratories. Examples of sharps include:
  - Hypodermic needles;
  - Syringes;
  - Pipettes;
  - Pipette tips;
  - Scalpel blades;
  - Blood vials;
  - Needles attached to tubing;
  - Broken and unbroken glassware;
  - Slides; and
  - Coverslips.

As a best management practice, EH&S manages discarded preparations made from genetically altered living organisms and their products as biohazardous waste. For example, recombinant DNA waste materials used in research laboratories is considered biohazardous waste.

## **H. Biohazardous Waste Handling**

Wastes associated with biological research materials must be disposed of in special ways. Examples of potentially hazardous items include:

- All sharps (e.g., glass implements, needles, syringes, blades, coming from facilities using infectious materials).
- Biologically-cultured stocks and plates.
- Agents (of any biosafety level) containing recombinant DNA.
- Human blood, tissues, organs, and cell lines.
- Transgenic plants and animals.
- Any regulated biohazardous material.

In order for the biological waste to be removed by EH&S, the following procedures must be followed:

- Biological wastes derived from human sources (e.g., blood, body fluids, tissues, tumors, human cell lines) are hazardous biological wastes and should be placed in a red or orange biohazard bag. Autoclave the bag under appropriate time and temperature and place it in a red drum for removal by hazardous waste personnel.
- BSL-1 materials containing recombinant DNA must be autoclaved or the DNA must be denatured with bleach.

- Bacteria, viruses, or other microorganisms that are known human pathogens should also be put into a red or orange bag, autoclaved, and placed in a red drum for removal by hazardous waste personnel.
- Sharps and sharp objects such as glass, syringes, disposable pipettes, and pipette tips that may be contaminated with biological waste (human or non-human) or pathogenic material should be placed in a rigid, leak-proof, puncture-resistant container. The container is autoclaved and then placed in a red drum for removal by hazardous waste personnel.
- Non-human biological wastes are handled by the same methods as human biological wastes: placed in autoclave bags, autoclaved, and put into red drums for removal by hazardous waste personnel.

When your “red-bag,” “yellow drum,” or liquid hazardous waste is ready for removal, call Environmental Management at (480) 965-3899 to arrange a pick-up, or submit a [hazardous waste pick-up request](#). If you need any other assistance or have questions, please feel free to contact Environmental Health & Safety at (480) 965-1823.

There are additional requirements for disposing of select agents and toxins. Please see the appendix titled “Special Considerations for Select Agents and Toxins.”

## **I. Biological Safety Audits**

EH&S audits laboratories at least once a year. The safety audit typically includes an evaluation of the autoclave, biological safety cabinet, microbiological techniques, emergency and safety equipment, storage of biohazardous material, general housekeeping, and review of the Lab-Specific Biosafety Manual. Please refer to the ASU Biosafety & Biosecurity [inspection checklists](#) for more information about the biosafety audit form used by EH&S.

EH&S will make every attempt to schedule laboratory audits with faculty members. However, if the principal investigator is unavailable or is unresponsive, EH&S will proceed with the safety audit. EH&S may also conduct unannounced accident investigations. Please be aware that federal, state, and local inspectors may also conduct unannounced inspections.

Following the biological safety survey, a report listing the safety concerns is sent to the faculty member responsible for the laboratory. The faculty member is responsible for correcting the hazards. If the faculty member fails to correct the hazard, a second notice is sent to the department head with a copy to the faculty member. Follow-up audits may be conducted in laboratories with extremely hazardous conditions and/or numerous violations.

### **III. RESPONSIBILITIES**

The biological safety program at ASU developed from the University's commitment to address and comply with the *NIH Guidelines* regarding safe research with recombinant DNA and associated biohazardous materials as well as the HHS and USDA regulations on select agents and toxins. The Institutional Biosafety Committee (IBC) and the department of Environmental Health & Safety (EH&S) provide oversight of ASU's biological safety program. The roles and responsibilities of ASU faculty and staff are described in this section.

#### **A. Arizona State University**

ASU has instituted and maintains a biosafety program for personnel who may be exposed to biological hazards (biohazards) during the performance of their duties. The biosafety program is designed to achieve regulatory compliance and to provide a means for employees to be informed about and protected from biohazards. To maintain regulatory compliance and to protect personnel from biohazards, Arizona State University must:

- Appoint a Biological Safety Officer (BSO) for the institution.
- Ensure appropriate training is provided to personnel conducting research with biohazardous or recombinant DNA materials.
- Ensure that research conforms to the provisions of the *NIH Guidelines*.
- Establish an Institutional Biosafety Committee with adequate expertise and training.
- Establish and maintain a health surveillance program for personnel.
- Implement policies for safe conduct of biological and recombinant DNA research.
- Report any significant problems, violations or significant research-related accidents or illnesses to the NIH OBA within 30 days.

#### **B. Institutional Biosafety Committee**

The Institutional Biosafety Committee (IBC) shall be constituted of a minimum of five (5) voting members. Collectively, the membership shall have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and identify any potential risks to public health or the environment. The IBC shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles and one scientist with expertise in animal containment principles. At least two members shall not be affiliated with the institution (apart from their membership on the committee) and shall represent the interest of the surrounding community with respect to public health and protection of the environment. The Biological Safety Officer (BSO) for the campus must be a voting member of the IBC. A quorum will consist of a simple majority. A passing vote will be a simple majority of members present; minority views will be recorded in the minutes. The committee's current membership may be obtained by contacting ORIA at <http://researchintegrity.asu.edu/>.

The responsibilities of the Institutional Biosafety Committee (IBC) include, but are not limited to, the following:

- Address biosafety issues related to experimentally infected laboratory animals.
- Adopt policies supporting the safe use of biological materials and the elimination or reduction of exposure to potentially biohazardous materials and agents.
- Assess the facilities, procedures, practices, training and expertise of personnel involved in research utilizing recombinant DNA and/or biohazardous materials.
- Determine necessity of health surveillance of personnel.
- Direct development of appropriate procedures to oversee the possession and/or use of recombinant DNA and biohazardous materials.
- Ensure training for IBC members, staff, Principal Investigators, and laboratory staff.
- Facilitate the registration of biological research by providing materials and information to Principal Investigators.



- Make a final determination of physical and biological containment for recombinant DNA and biohazardous materials, and modify containment levels as necessary.
- Notify the Principal Investigator of the results of the IBC's review, approval, or disapproval.
- Obtain specific review, registration and/or approval from NIH/OBA for research that falls under **Sections III-A, III-B, III-C** and **Appendix M** of the *NIH Guidelines*.
- Review and report any significant problems, violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the Institutional Official and to the appropriate authorities.
- Review, approve and oversee research utilizing recombinant DNA and biohazardous materials, conducted at or sponsored by the University, for adherence with the *NIH Guidelines*, the BMBL, and University policies. This pertains to the initial and continuing reviews and modifications to the currently approved research.
- Review and approve research involving the use of select agents and toxins.
- Review research involving recombinant DNA and other potentially biohazardous agents.
- Set biosafety containment levels as required by the *NIH Guidelines*.
- Suspend or terminate disclosure approval for the possession or use of recombinant DNA and biohazardous materials, where the IBC finds non-compliance or that such use or possession poses undue risk to research personnel or a threat to the health and safety of the community.
- Periodically review the IBC policies and procedures and modify them as necessary to ensure appropriate biosafety measures and adherence with federal and state requirements.
- Review research disclosures that include the possession and/or use of recombinant DNA and biohazardous materials for compliance with *NIH Guidelines*. As part of the review process, the IBC will do the following:
  - Conduct an independent assessment of the containment levels (i.e., BSL-1, BSL-2, BSL-3) in collaboration with EH&S, as required by the *NIH Guidelines*.
  - Conduct an assessment of the facilities, procedures, practices, training, and expertise of personnel conducting research involving recombinant DNA and biohazardous materials.
  - Ensure adherence with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines* for recombinant DNA.
- Submit membership roster (completed by the ASU ORIA) including members' biographical sketches to the NIH Office of Biotechnology Activities (OBA).

According to the *NIH Guidelines*, the IBC must review and approve architectural designs for new facilities and renovations to existing facilities where recombinant DNA research may be performed. The IBC has delegated the authority to EH&S to review and approve these documents on their behalf.

### **C. Department of Environmental Health & Safety/Biosafety Officer**

The responsibilities of EH&S and the Biological Safety Officer (BSO) include, but are not limited to, the following:

- Advise researchers on proper waste disposal methods based on federal and state regulations.
- Assist in the development of emergency plans for handling accidental spills and personnel contamination.
- Consult with researchers on issues of biosafety and the safe use of biological materials in the laboratory.
- Investigate laboratory accidents involving recombinant DNA research.
- Develop protocols and procedures to address issues of biosafety.

- Develop, implement, and maintain the university's biosafety program.
- Develop, implement, and maintain the university's program for select agents and toxins.
- Perform periodic inspections to ensure that laboratory standards are rigorously followed.
- Promote regulatory compliance and a safe laboratory environment.
- Provide advice on laboratory security.
- Provide oversight of the ASU [Bloodborne Pathogen Program](#) and conduct training for laboratory personnel with such exposure.
- Provide technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.
- Provide training in the safe use and practices for those working with potentially biohazardous materials and activities.
- Report to the Institutional Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator.
- Work in conjunction with the IBC and the ORIA to review all registration forms for research proposals submitted by Principal Investigators.

**D. Office of Research Integrity and Assurance**

- Accepts all IBC forms from PIs for research involving any of the following: 1) recombinant DNA, 2) biohazardous agents, and 3) select agents and toxins, and coordinates their review with the IBC and EH&S.
- Reviews biological Material Transfer Agreements (MTA) and coordinates approval with EH&S.
- Accepts research protocols involving the use of animals and coordinates review by the Institutional Animal Care and Use Committee (IACUC).
- Accepts research protocols involving the use of human subjects and coordinates review by the Institutional Review Board (IRB).

**E. Deans, Directors, and Chairs**

- Ensure that registration forms are completed by each Principal Investigator conducting research involving biohazardous materials (microorganisms, microbial toxins, or other biological agents that can infect and/or cause disease in humans, animals, or plants), human blood, body fluid, tissues, and cell lines of human origin, genetically-modified organisms, and recombinant DNA molecules.

**F. Responsible Official**

A Responsible Official (RO) is required under the HHS and USDA select agent regulations. This individual's responsibilities can be found in the applicable regulations. Although this list is not intended to be a complete list, critical responsibilities include:

- Developing and implementing safety, security, and emergency response plans.
- Allowing only approved individuals to have access to select agents or toxins.
- Providing appropriate training for safety, security and emergency response.
- Transferring select agents or toxins only to registered individuals.
- Providing immediate notice of any theft, loss, or release of a select agent or toxin.
- Providing proper laboratory facilities to contain and dispose of select agents and toxins.
- Maintaining complete records relating to select agents as defined in 42 CFR 73.15.
- Reporting the identification of a select agent or toxin as a result of diagnosis, verification, or proficiency testing.
- Submitting changes in the registration information by promptly notifying the CDC or Animal and Plant Health Inspection (APHIS)/USDA in writing. This includes modifications

to the list of individuals that have been approved to work or access select agents, changes in work locations, and changes in protocols or objectives of the studies.

- Conducting regular inspections, at least annually, of the laboratory where select agents or toxins are stored or used to ensure compliance with all procedures and protocols of this safety plan. The results of these inspections must be documented and any deficiencies must be corrected and documented.

**The RO for Arizona State University is:**

- Leon C. Igras, Director, EH&S

**The Alternate Responsible Officials (ARO) for Arizona State University is:**

- David Gillum, Associate Director of Biosafety/Biosecurity, EH&S
- Steven Hunter, Associate Director, EH&S
- Irene Mendoza, Assistant Biosafety Officer, EH&S

**G. Principal Investigator**

A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling biohazardous agents must be responsible for the conduct of work with any biohazardous agents or materials. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment. Responsibilities of the Principal Investigator (PI) include:

- Accept direct responsibility for the health and safety of those working with biohazardous materials and/or select agents and toxins in the laboratory.
- Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination.
- Be adequately trained in good microbiological techniques.
- Comply with shipping requirements for biohazardous.
- Develop specific biosafety standard operating procedures for biohazardous materials used in the laboratory.
- Ensure compliance by laboratory personnel with relevant regulations, guidelines, and policies.
- Ensure personal protective equipment is provided and used.
- Ensure proper training and instruction for laboratory personnel in safe practices and protocols, including, at a minimum, training in aseptic techniques and biology of the organism(s) used. Please refer to the ASU [Lab-Specific Biosafety Training Checklist](#).
- Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity, genotypic and phenotypic characteristics) through annual testing and certification.
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.
- Provide to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken.
- Remain in communication with the IBC throughout the conduct of the project.
- Report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety and other authorities, as appropriate.
- Submit a disclosure to the Institutional Biosafety Committee (IBC) for review and approval or disapproval.

- Supervise laboratory staff to ensure that the required safety practices and techniques are employed. Correct work errors and conditions that may result in accidents, injuries, or the release of biohazardous materials.

PIs are also responsible for full compliance with the *NIH Guidelines* during the conduct of recombinant DNA research.

- Determine whether the recombinant DNA research is subject to the *NIH Guidelines*.
- Develop specific biosafety standard operating procedures for recombinant DNA materials used in the laboratory.
- Obtain IBC approval before initiating recombinant DNA research subject to the *NIH Guidelines*.
- Petition OBA, with notice to the IBC, for proposed exemptions from the *NIH Guidelines*.
- Propose physical and biological containment levels in accordance with the *NIH Guidelines* when registering research with the IBC.
- Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer, Greenhouse/Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other authorities, as appropriate, within 30 days.
- Seek OBA approval, in addition to IBC approval, to conduct experiments specified in **Sections III-A** and **III-B** of the *NIH Guidelines*.
- Seek the NIH Office of Biotechnology's (OBA) determination of containment for experiments that require a case-by-case review.
- Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the disclosure to the IBC for review and approval or disapproval.

#### **H. Laboratory Employees**

- Participate in appropriate training and instruction and ensure that they are adequately trained and fully understand the instructions.
- Fully comprehend all biological agents and select agents and toxins being used in the lab and the potential risks associated with exposure, as well as fully understanding the associated emergency response procedures.
- Follow all laboratory practices and protocols and comply with all applicable policies, procedures, and guidelines.
- Complete any necessary medical surveillance.
- Report all thefts, security incidents, accidents, spills, or contamination incidents to supervisor.

#### **I. Institutional Animal Care and Use Committee**

The Institutional Animal Care and Use Committee (IACUC) works in cooperation with the Occupational Health Nurse, the Director of the Department of Animal Care and Technologies (DACT), and the University Veterinarian, to advise investigators and animal care personnel on the potential biohazards and risk of physical injury associated with laboratory animals and on the procedures for reducing or eliminating exposure. It also ensures compliance with all applicable regulations, policies, and procedures based on the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

- DACT ensures that biosafety cabinets in animal facilities are certified as needed.
- DACT and the University Veterinarian provide training to all animal users in proper methodology and safe animal handling. ORIA ensures that all employees receive appropriate training and maintains those records.

## **J. Other Organizations**

Other committees, including the Human Subjects Committee, Radiation Safety Committee, and the Department of Public Safety must:

- Consult and coordinate with the IBC and EH&S on any proposals under their purview which involve the use of potentially biohazardous materials or activities.

## **K. Visitors, Vendors, and Contractors**

All visitors, vendors, and contractors must:

- Comply with all security requirements and procedures.
- Be accompanied by a Department of Justice approved person at all times while in areas with select agents or toxins; and
- Use personal protective equipment provided for them by the laboratory.

**NOTE:** Contractors must ensure that appropriate personal protective equipment is available for their own workers.

## **IV. INCIDENT REPORTING**

### **A. Reportable Incidents and Violations**

Incidents or problems involving recombinant DNA and/or biohazardous materials must be immediately reported to the Biological Safety Officer (BSO). Examples of reportable significant incidents include but are not limited to any overt exposure, such as a needle stick, splash, and contamination due to equipment failure, and any potential exposure to biohazardous materials. A significant event may also occur from a containment breach, which may be subsequently determined to pose either an overt or potential exposure to individuals. It should be noted that waste from recombinant DNA research is considered biohazardous and incidents involving improper disposal of recombinant DNA must also be reported. Questions regarding reportable incidents should be directed to the BSO.

Failure by research personnel to follow federal and institutional regulations, guidelines, policies and/or procedures may also require reporting to the appropriate institutional, local, state and/or federal agencies. Violations may include but are not limited to conduct of new or ongoing research without appropriate federal or institutional registration, review, approval or oversight.

### **B. Principal Investigator Responsibilities**

The Principal Investigator and their personnel must report any significant incident, violation of the *NIH Guidelines*, or any significant, research-related accidents and illnesses immediately by contacting the BSO. Examples of incidents and violations include:

- Overt exposures, which are defined as exposures that result in direct personnel exposure to biohazardous materials such as injection, spills, splashes or aerosol inhalation.
- Potential exposures, which are defined as exposures that have a high risk of exposing personnel to biohazardous materials such as spills, containment failure while working with the agent or equipment failure that may produce aerosols.
- Any exposure (overt or potential) in a BSL-3 laboratory.
- Overt exposure in BSL-1 or BSL-2 laboratories.
- Any illness that may be caused by the agents used in the laboratory.
- Any incident involving the improper disposal of recombinant DNA.

### **C. Biosafety Officer Responsibilities**

The Biological Safety Officer (BSO) is required, by the *NIH Guidelines*, to report to the IBC:

- All violations of the *NIH Guidelines* and significant incidents.
- Any significant research-related accidents or illnesses.

### **D. Institutional Responsibilities**

The IBC is required, by the *NIH Guidelines*, to report to the appropriate University official and to the NIH/OBA within 30 days any significant incidents, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses. The IBC will be responsible to determine what actions, if any, are necessary. For example the IBC may choose to change the frequency of lab inspections, or change the biosafety level of the disclosure, based on results of the incident.

Other IBC reporting requirements (to OBA and other agencies) include but are not limited to:

- Research involving recombinant DNA or biohazardous materials without prior IBC approval.
- Lax security, unsafe procedures used in a laboratory setting, improper disposal of recombinant waste.

- Significant changes to proposed research risk without prior notification and approval by IBC.

Some incidents must be reported to OBA on an expedited basis. Spills or accidents in BSL-2 laboratories (involving recombinant DNA) resulting in an overt exposure must be immediately reported to OBA. In addition, spills or accidents involving recombinant DNA occurring in high containment (BSL-3 or higher) laboratories resulting in an overt or potential exposure must be immediately reported to OBA. The IBC will report to the appropriate institutional official, who, in turn will report to OBA, any of the above-described incidents.

Institutional violations that will be reported to the appropriate college or department head may include but are not limited to:

- Lapses in disclosure approval.
- Failure to comply with institutional and federal regulations, guidelines, and policies.
- Unsafe work practices.

#### **E. Institutional Official Responsibilities**

Upon receiving a report from the IBC, the Institutional Official (IO) will directly report:

- In writing any problems with or violations (non-compliance) of the *NIH Guidelines*, or any significant incident, accidents, or illnesses related to recombinant DNA, to the NIH/OBA within 30 days or immediately for overt exposure to a BSL-2 agent or potential/overt exposure to a BSL-3 agent.
- Any significant research-related illness or accident that may be hazardous to the public health and cooperate with state and local public health departments.

## V. **BIOHAZARDOUS RESEARCH PROJECT REGISTRATION AND APPROVAL**

### A. **Introduction**

Each PI is responsible for the preparation of registration documents for all research involving potentially biohazardous materials, including the assignment of the required Biological Safety Level (BSL) to the proposed biological research. Biological research includes research involving:

- Bacterial, viral, fungal, parasitic, or other potentially infectious agents.
- Recombinant DNA, including experiments that may be exempt under the *NIH Guidelines*.
- Human blood, body fluid, and tissue, including human cell lines.
- Listed select agents and toxins.

The IBC, in conjunction with EH&S, will review all submitted registration documents; confirm, where applicable, that exempt status is appropriate for certain recombinant DNA work; and consider approval for those registration documents that are complete and that provide for safe handling of potentially biohazardous materials under the appropriate BSL.

### B. **Registration and Approval Process**

Additional requirements referred to in the registration documents for research involving select agents or toxins, infectious materials, or human blood or tissue (as outlined below) will need to be fulfilled for final approval to be given.

**If the project involves or may involve recombinant DNA; infectious agents (other than select agents and toxins); human blood, tissue or cell cultures; or other biosafety concerns, the following steps must occur:**

1. The Office of Research Integrity and Assurance (ORIA) or the Biosafety Officer distributes registration forms and the ASU Biosafety Manual to anyone requesting them and to other researchers who may work with recombinant DNA, infectious agents, human samples, or other biosafety concerns.
2. The Principal Investigator (PI) or researcher reviews the ASU Biosafety Manual and, if applicable, completes [Form 112](#) for recombinant DNA, infectious agents, human blood, and tissue or cell culture. The PI may contact ORIA or the Biosafety Officer in EH&S to discuss the project or for guidance in completing the forms. In addition, to assist the PI in determining the appropriate biosafety level for the proposed research, the PI is directed to the [Agent Summary Statements in the BMBL](#) and Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard in the *NIH Guidelines*.
3. The PI submits the completed application and necessary attachments to ORIA.
4. ORIA reviews the submitted application for completeness and may contact the PI for additional information.
5. The Biosafety Officer and members of the Institutional Biosafety Committee review the completed application to determine whether the project meets federal and university standards.
6. At its monthly meeting, the IBC discusses the proposed project and votes to approve the research as presented, to approve it with stipulations, or to table it for further information.
7. If the project is approved, the Chair of the IBC signs the application. ORIA retains all administrative records.



8. ORIA, on behalf of the IBC, will notify the PI of the status of the application, including any stipulations or comments for resolution.

**Additional Notes:**

- A "Continuing Review to Original 112AB Disclosure" [Form 112AR](#) is available to facilitate the annual renewal process.
- If the research involves any listed select agents or toxins, the PI must submit [Form 112C](#) as an initial starting point.
- Copies of the 112 application forms can be downloaded from the ORIA website at <http://researchintegrity.asu.edu/biosafety/forms> or can be obtained the Biosafety Officer through EH&S.

**C. Additional Information and Requirements**

**1. Select Agents and Toxins**

All personnel at Arizona State University are prohibited from possessing, using, receiving, or transferring listed select agents or toxins unless ASU has been granted a certificate of registration by the HHS Secretary or the USDA Secretary. Additional requirement for Select Agents and Toxins are found in the appendix titled "Special Considerations for Select Agents and Toxins."

**2. Human Blood and Tissue**

In any laboratory where work involves the use of and/or exposure to human blood, body fluids, or unfixed human tissue, including human cell cultures, there is the danger of exposure to bloodborne pathogens (disease-causing microorganisms) that may be found in such material. Work with any of these materials in a laboratory setting must follow ASU procedures as described in the [ASU Exposure Control Plan](#).

In addition, when human blood or tissue donors are involved, the PI must contact ORIA to determine whether a human subject Institutional Review Board (IRB) application is required. The IRB Coordinator may be contacted in ORIA.

**3. Animal Handling**

When research involves exposure to and handling of animals, consideration must be given to the potential allergens, animal pathogens, zoonoses, and physical hazards, e.g., bites and scratches that may be encountered by researchers and staff. All research involving the use of animals conducted under the auspices of ASU is reviewed by the [Institutional Animal Care and Use Committee](#) (IACUC), in compliance with federal regulations. Projects involving animal research require that applications be submitted to the IACUC for protocol approval before research begins.

Principal Investigators and all personnel involved in the care and use of research animals must obtain certification in the [ASU's Humane Practice of Animal Care and Use Training Program](#). All of these requirements must be fulfilled prior to the acquisition and use of laboratory animals, including medical review with the Occupational Health Nurse at ASU Campus Health Services.

#### **4. Recombinant DNA Materials**

The use of recombinant DNA is regulated by the NIH, as outlined in the *NIH Guidelines*. At ASU this research must be reviewed by the IBC prior to initiation of the work. Guidelines include registration of the recombinant DNA, understanding the classification of the use of recombinant DNA, and safe work practices/proper disposal of material (including whole animals) containing recombinant DNA. The use of more than 10 liters of recombinant material requires special practices.

#### **5. Environmental Samples**

Environmental samples, such as water, air, or earth, may contain pathogens (i.e., bacteria, viruses, spores) that could present a health hazard to people, animals, or the environment. Using appropriate personal protective equipment when collecting environmental samples will reduce exposure to potential pathogens. Use care when handling environmental samples, especially if the sample will be enhanced in the laboratory by culturing or other growing mechanisms. Techniques used to enhance and/or culture environmental samples should be conducted at BSL-2 or higher levels in an appropriate containment device, such as a biological safety cabinet or fume hood. If the environmental sample is sterilized prior to experimentation, then the sample may be manipulated in a BSL-1 rated laboratory.

Research involving environmental samples must be registered with the ASU Institutional Biosafety Committee (IBC) for approval. If you require assistance determining whether you need to register this research with the IBC, please contact the Biological Safety Officer.

#### **6. Protective Clothing Beyond the Laboratory**

The improper use or lack of protective clothing and equipment in a laboratory can lead to chemical burns, biological exposures, or other potential dangers. To help reduce the risk of exposure, personnel in ASU laboratories are required to wear gloves, safety glasses, lab coats and other personal protective clothing. However, in public areas, such as hallways and lounges, wearing personal protective clothing and equipment is not recommended. This is because contaminated clothing may present a hazard, and the perception of contaminated protective clothing and equipment in a public area may project a careless image to both colleagues and visitors.

Wearing gloves outside the laboratory should be minimized, except to move hazardous materials between laboratories. Chemicals should be transported from place to place on a cart, in a clean secondary container, or in a bottle carrier with secure handles. When this is not an option, personnel should use a clean, ungloved hand to touch common surfaces and a gloved hand to carry the items: the one-glove rule. Alternatively, the material should be packaged so the outer container may be transported without the need for personal protective equipment.

Protective gloves should never come into contact with door handles, elevator buttons, telephones, lavatory faucets, vending machines, bottled water dispensers, ice-making machines, or other surfaces outside the laboratory. Also, please be aware that strict federal and state regulations address the transport of hazardous (i.e., biological, chemical, radiological) materials on public roads.

For the sake of safety, appearances, and courtesy, personnel are asked not to wear contaminated, stained, or potentially contaminated lab coats and other research clothing and equipment in any public area, especially dining areas, lounges, auditoriums, conference rooms, or other non-hazardous areas.

## 7. Food and Beverages in the Laboratory

In order to reduce potential exposures and to ensure compliance with prudent laboratory operations, regulations, and other best management practices, ASU prohibits the storage and consumption of food and drink in all campus laboratories. The only exception is for food and beverages used in research and teaching projects. These materials must be labeled, "Not for Human Consumption."

In order to prevent potential exposure to hazardous materials:

- Do not eat, drink, smoke, chew gum, apply cosmetics, or take medicine in laboratories where hazardous materials are handled or stored.
- Do not store food, beverages, cups, or other drinking and eating utensils in areas where hazardous materials are handled or stored.
- Do not use glassware for laboratory operations to prepare or consume food or beverages.
- Do not use laboratory refrigerators, ice chests, cold rooms, and ovens for food storage or preparation.
- Do not use laboratory water sources or deionized laboratory water for drinking water.

**Important:** Food and beverages must never be stored in any laboratory refrigerator in which chemicals, biological, and radioactive materials are kept unless they have been labeled, "Not for Human Consumption."

## **VI. TRAINING FOR WORKING SAFELY WITH BIOHAZARDOUS AGENTS**

The PI or laboratory supervisor is responsible for providing or arranging for site-specific training of all personnel. In addition, each employee must attend Biosafety Training and Laboratory Chemical Safety Training. Contact EH&S or the Biosafety Officer for more information on scheduling training. Training must be documented. Please refer to the [ASU Laboratory-Specific Training Checklist](#) for more information.

## VII. LABORATORY PROCEDURES AND EQUIPMENT

### A. Exposure Control

The term “containment” is used in describing safe methods for managing biohazardous and select agents and toxins in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The three elements of containment include laboratory practices and techniques, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements. Each PI is required to complete a Health Hazard Assessment for each biological agent and toxin stored in his or her laboratory (see [Appendix B](#) of this manual). Copies of the Health Hazard Assessments must be included in the PI’s Lab-Specific Biosafety Manual.

#### 1. Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for handling such material safely. The PI of each laboratory is responsible for providing or arranging the appropriate training of personnel and for verifying each person’s competence. In addition, each PI must develop a Lab-Specific Biosafety Manual to address the use, handling, and disposal of biohazardous material (including select agents and toxins) in the laboratory. The Lab-Specific Biosafety Manual must identify specific hazards that will or may be encountered and consider procedures needed to minimize or eliminate risks. Personnel should be advised of special hazards and are expected to follow the required practices and procedures. A template for the Lab-Specific Biosafety Manual may be found in Appendix B of this manual.

#### 2. Safety Equipment (Primary Barriers)

Safety equipment includes biological safety cabinets (BSC), enclosed biohazardous containers, and other engineering controls designed to eliminate or minimize exposures to biohazardous agents and toxins. The biological safety cabinet is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. More information on BSCs may be found in the “*Biological Safety Cabinet*” section of this manual.

Primary safety barriers may also include personal protection equipment (PPE) such as gloves, lab coats, safety glasses or goggles, face shields and respirators. Personal protective equipment is often used in combination with BSCs and other containment devices. In some situations in which it is impractical to work in a BSC, personal protective equipment may form the primary barrier between the worker and the infectious materials. **ASU policy requires the use of gloves, lab coat and eye protection at all times when handling biohazardous materials.**

### 3. Facility Design (Secondary Barriers)

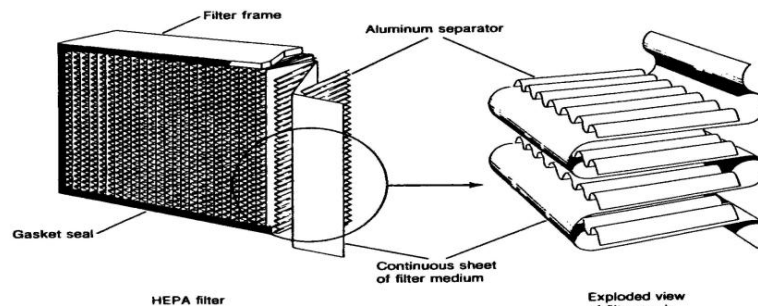
The design of a facility is important in providing a barrier to protect those working inside and outside the laboratory and to protect people or animals in the community from biohazardous agents and toxins which may be accidentally released from the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The secondary barriers required will depend on the risk of transmission of specific agents. For example, in working with agents at Biosafety Level 2, the exposure risks involve direct contact with the agents or inadvertent contact through contaminated work environments. Recommended secondary barriers in these laboratories include separation of the laboratory work area from public access, handwashing facilities, and availability of a decontamination facility such as an autoclave.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional airflow, HEPA filtration to remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate modules to isolate the laboratory.

#### B. Biological Safety Cabinets (BSCs)

BSCs are classified as Class I, Class II, or Class III cabinets. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air) filters (See Figure 1. Source: Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition, Appendix A). Biosafety cabinets should not be confused with clean benches which only protect the material being worked with and are not suitable for work with infectious or toxic material. (Although clean benches, like BSCs, have HEPA-filtered air, in clean benches the air flows over the experimental material toward the user rather than being drawn away.) BSCs should also not be confused with conventional fume hoods that do not filter microorganisms.

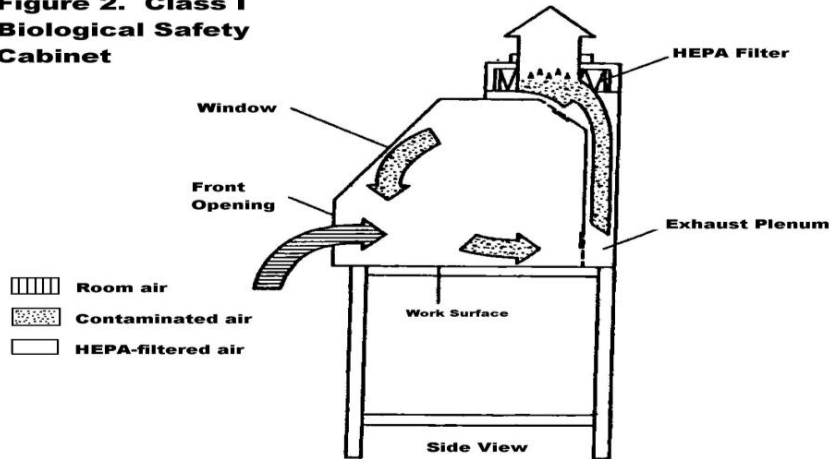


**Figure 1. Diagram of HEPA filter. These filters are typically constructed of continuous sheets of paper-thin filter medium, pleated to increase surface area, divided by aluminum separators, and affixed to a frame.**

**Types of BSCs:**

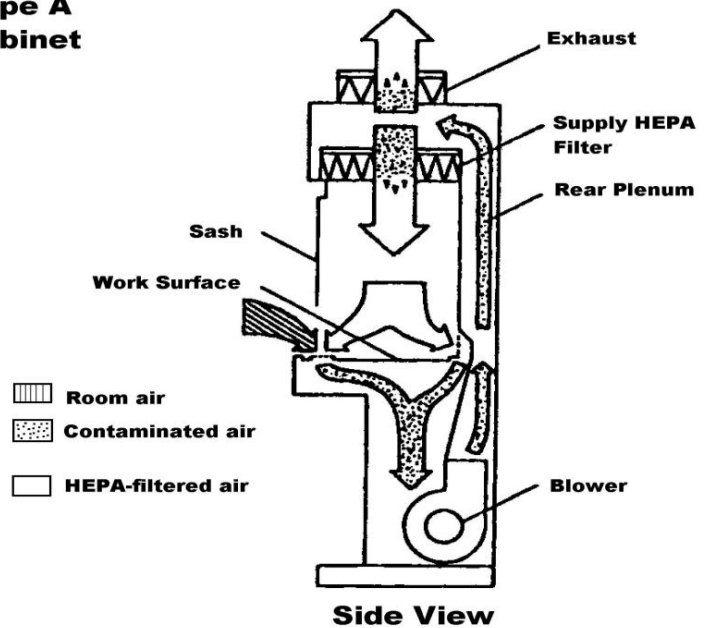
**Class I BSCs** provide personnel and environmental protection, but not product protection (See Figure 2. Source: *Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition, Appendix A*).

**Figure 2. Class I Biological Safety Cabinet**



**Class II BSCs** are the most commonly used BSC at ASU for biohazardous agents. These cabinets provide personnel, environmental, and product protection (See Figure 3. Source: *Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition, Appendix A*). Only those which are hard ducted to the outside and provide a face velocity of 80 to 125 feet per minute should be used when working with volatile chemicals. Additionally, cabinets are not designed to prevent ignition of volatile flammable chemicals, such as ethanol and isopropanol.

**Figure 3. Class II, Type A Biological Safety Cabinet**



### **Guidelines for Working in a BSC**

1. Turn off the ultraviolet lamp if one is in use. Turn on the fluorescent lamp.
2. Inspect the air intake grilles for obstructions and foreign material and remove if necessary.
3. Adjust view screen to proper height.
4. Turn the cabinet on for at least 10 minutes prior to use, if the cabinet is not left running.
5. Prepare a written checklist of materials necessary for the particular activity.
6. Wash hands and arms with mild soap. Put on a rear-fastening, long-sleeved gown with tight-fitting cuffs. Put on safety glasses and a pair (or two pairs) of high quality nitrile gloves.
7. Disinfect work surface with a suitable disinfectant.
8. Place items into the cabinet so that they can be worked with efficiently without unnecessary disruption of the airflow, working with materials from the clean to the dirty side.
9. Adjust the working height of the stool so that the worker's face is above the front opening.
10. Delay manipulation of materials for approximately one minute after placing hands/arms inside the cabinet.
11. Minimize the frequency of moving hands in and out of the cabinet.
12. Do not disturb the airflow by covering any portion of the grillwork with materials.
13. Work at a moderate pace to prevent the air flow disruption that occurs with rapid movements.
14. Wipe the bottom and side of the hood surfaces with disinfectant when work is completed.

**NOTE:** Be very careful when using small pieces of materials such as chemwipes in the hood. These can be blown into the hood and disrupt the motor operations.

### **Certification of the BSC**

Certification is a series of performance tests on the BSC to confirm that it will provide the user and experimental material the protection for which it is designed. The airflow, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards. Certification is arranged through each department and provided by an outside vendor.

BSCs intended for research with biohazardous materials must be certified:

- After they are received and installed (before use with infectious materials).
- After filter changes.
- After being moved (even a few feet).
- Annually.

BSC decontamination (using the formaldehyde gas production process) must be provided (e.g., by an outside vendor) and needs to be done:

- Before any maintenance work requiring disassembly of the air plenum, including filter replacement.
- Prior to cabinet recertification.
- Before moving the cabinet to a new laboratory.
- Before discarding or salvaging.

The production of formaldehyde gas is a health concern. Many BSCs at ASU are not ducted to the outside; therefore, extreme caution should be used when having the procedure performed.



## **VIII. GUIDELINES FOR GOOD LABORATORY PRACTICES**

### **A. Guidelines for Good Laboratory Practices at BSL-1**

BSL-1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

#### **1. Standard Microbiological Practices**

- a) The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- b) Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- c) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- d) Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- e) Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - iii. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- f) Perform all procedures to minimize the creation of splashes and/or aerosols.
  - g) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
  - h) Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

- i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport
  - ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- i) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
  - j)
  - k) An effective integrated pest management program is required.
  - l) The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## **2. Special Practices**

None required.

## **3. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

- a) Special containment devices or equipment, such as BSCs, are not generally required.
- b) Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- c) Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
- d) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment.
- e) Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
  - i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
  - ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

## **4. Laboratory Facilities (Secondary Barriers)**

- a) Laboratories should have doors for access control.
- b) Laboratories must have a sink for hand washing.

- c) The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
  - iii. Laboratories windows that open to the exterior should be fitted with screens.

## **B. Guidelines for Good Laboratory Practices at BSL-2**

BSL-2 builds upon BSL-1 practices. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

### **1. Standard Microbiological Practices**

- a) The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- b) Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- c) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- d) Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- e) Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- iii. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

- iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- f) Perform all procedures to minimize the creation of splashes and/or aerosols.
- g) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- h) Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- i) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
- j) An effective integrated pest management program is required.
- k) The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## **2. Special Practices**

- a) All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- b) Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- c) Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
- d) A Lab-Specific Biosafety Manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- e) The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- f) Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- g) Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

- i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- h) Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- i) Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- j) All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

### **3. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

- a) Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:
- i. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
  - ii. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- b) Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
- c) Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
- d) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
- a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

- c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- e) Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

#### **4. Laboratory Facilities (Secondary Barriers)**

- a) Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
- b) Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.]
- c) The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
- d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- e) Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
- f) BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
- g) Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- h) An eyewash station must be readily available.
- i) There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
- j) HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
- k) A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

#### **C. Guidelines for Good Laboratory Practices at BSL-3**

BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment. A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

### **1. Standard Microbiological Practices**

- a) The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- b) Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- c) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- d) Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- e) Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - iii. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- f) Perform all procedures to minimize the creation of splashes and/or aerosols.
  - g) Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
  - h) Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    - i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

- ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- i) A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
- j) An effective integrated pest management program is required.
- k) The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## **2. Special Practices**

- a) All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- b) Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- c) Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
- d) A Lab-Specific Biosafety Manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- e) The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
- f) Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- g) Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- h) Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- i) Animals and plants not associated with the work being performed must not be permitted in the laboratory.



- j) All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

### **3. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

- a) All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
- b) Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls is worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
- c) Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
- d) Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:
  - i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- e) Eye, face, and respiratory protection must be used in rooms containing infected animals.

### **4. Laboratory Facilities (Secondary Barriers)**

- a) Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
- b) Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
- c) The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

- i. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
- ii. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
- iii. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

- d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
  - i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- e) All windows in the laboratory must be sealed.
- f) BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
- g) Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- h) An eyewash station must be readily available in the laboratory.
- i) A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
  - i. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
  - ii. The laboratory exhaust air must not re-circulate to any other area of the building.
  - iii. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
- j) HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

- k) A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
- l) Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
- m) Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
- n) Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
- o) The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

## IX. **EMERGENCY RESPONSE PROCEDURES**

ASU has a campus-wide emergency response plan, the ASU Emergency Operations Plan, which is compliant with 29 CFR 1910.120. Protocols for handling biological emergencies are outlined in the plan. A summary of this plan can be found by reviewing the [ASU Emergency Procedures Flipchart](#) or the [ASU Police Department Policies and Procedures Manual](#).

Principal Investigators must be aware of the provisions for emergency procedures and preparedness. Emergency procedures and preparedness must be incorporated into the Lab-Specific Biosafety Manual and used in the laboratory. Each laboratory should have a written emergency plan specifying the appropriate response to potential emergencies. Accidents and spills of infectious materials will be discussed in Emergency Procedures below.

In addition, each PI will submit to EH&S the following:

- A completed [Responsible Party Information Sheet](#);
- An annual chemical and biological inventory; and
- A Health Hazard Assessment for each biological agent and toxin stored or used in the laboratory. (See Appendix B of this manual.)

### A. **Decontamination**

Decontamination is a process or treatment that renders an instrument or environmental surface safe to handle. A decontamination procedure can be as simple as clean-up with detergent and water or as thorough as sterilization.

- **Sterilization** is the use of physical or chemical processes to destroy all viable forms of microbial life, such as bacterial spores.
- **Disinfection** is a chemical or physical treatment that destroys the most resistant vegetative microbes or viruses, but not the spores, in or on inanimate surfaces. Effectiveness is influenced by a number of factors, including the type and number of organisms, amount of organic matter, the object being disinfected, the disinfectant being used, concentration, temperature and exposure time.
- **Antisepsis** is the application of a liquid antimicrobial to skin or other living tissue to inhibit or destroy microorganisms. Examples include handwashing with germicidal solutions or swabbing skin with alcohol before an injection.

Sterilization, disinfection, and antisepsis are all forms of decontamination.

#### 1. **When to Decontaminate**

All material and equipment contaminated with or containing potentially biohazardous agents should be decontaminated:

- Upon completion of procedures involving the use of biohazardous material;
- In the event of spills of biohazardous materials;
- Before being washed, stored, or discarded; and
- At least daily.

In most ASU laboratories, it is recommended that decontamination be accomplished by steam heat sterilization in an autoclave or by surface application of or placement in a chemical disinfectant solution, such as 1:10 bleach solution or its equivalent.

## 2. Autoclave Use

Autoclaving (saturated steam under pressure of approximately 15 pounds per square inch (psi) to achieve a chamber temperature of at least 250°F for a designated time) is the preferred and most convenient method to rapidly destroy all forms of microbial life. However, to do this, the autoclave process must reach proper temperature, pressure, and time, and also prevent the entrapment of air in the bag or container of treated material.

- Material to be sterilized must come into contact with live steam.
- Bags or containers should be left open during autoclaving or water (~200 ml) should be added to sealed bags to generate steam.
- Heat indicator tape should be used inside the bag or container with each autoclave load to indicate that sterilization has been completed.
- Autoclave sterility monitoring should be conducted on a regular basis using biological indicators (such as *B. stearothermophilus* spore strips) placed among treated materials and at locations throughout the autoclave. The spores, which are more resistant to heat than most other biological materials, provide validation of general microbial destruction when they are effectively inactivated by autoclave operation (typically 250°F for 30 minutes).

## 3. Chemical Disinfectant Use

The most practical use of chemical disinfectants is for surface decontamination and, when used in sufficient concentration, as a decontaminant for liquid wastes prior to final disposal. General recommendations are:

### ***Liquid Decontamination***

- Add liquid chlorine bleach to provide a final 1:10 dilution;
- Let stand at least 20 minutes; and
- Discard the solution appropriately.

### ***Surface Decontamination***

- Wipe with 1:10 dilution of chlorine bleach; or
- Wipe with iodophor disinfectant (per label concentration).

See [Table 1](#), "Disinfectant Activity" at the end of this manual for further information on disinfectants.

## B. Exposure to Biohazards

In the event of an exposure to a biohazardous agent or material, the following guidelines should be used:

### **Intact Skin**

1. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
2. Vigorously wash contaminated skin for 1 minute with soap and water.
3. Call 911 or seek medical attention at the ASU Health Services, if necessary.
4. Inform the laboratory's PI and EH&S.

### **Broken, Cut or Damaged Skin or Puncture Wound**

1. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
2. Vigorously wash contaminated skin for 5 minutes with soap and water.
3. Call 911 or seek medical attention at the ASU Health Services, if necessary.
4. Inform the laboratory's PI and EH&S.

### **Eye**

1. Immediately flush eyes for at least 15 minutes with water, using an eyewash. Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.
2. Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
3. Call 911 or seek medical attention at the ASU Health Services, if necessary.
4. Inform the laboratory's PI and EH&S.

### **Ingestion or Inhalation**

1. Move to fresh air immediately.
2. Call 911 or seek medical attention at the ASU Health Services, if necessary.
3. Do not induce vomiting unless advised to do so by a health care provider.
4. Inform the laboratory's PI and EH&S.

## **C. Spills of Biohazards**

ASU does not have a centralized biological spill response team. Therefore, each laboratory working with potentially hazardous biological material must be prepared and trained to handle its own biological spills. EH&S is available for assistance if necessary. Additional information pertaining to spills which involve recombinant DNA, blood, microorganisms, or any other bioresearch materials can be found at <http://cfo.asu.edu/emergency>.

Performing all work on plastic-backed liners to absorb spills can minimize the consequences of a spill of a biohazardous or select agent. The quantities of these materials should be limited so they can be easily contained, cleaned, or destroyed. If respiratory protection is required, the [ASU Respiratory Protection Program](#) must be followed. A simple spill kit should be on hand including:

- Chlorine bleach or some other concentrated disinfectant
- A package or roll of paper towels
- Autoclave bags
- Rubber gloves
- Forceps for picking up broken glass.

## **D. Spills Inside a Biological Safety Cabinet (BSC)**

1. Alert the other laboratory employees.
2. **Leave the cabinet turned on.**
3. While wearing gloves, spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
4. Report the spill to the laboratory's PI, who will report the spill to the RO if a select agent or toxin is involved.

5. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
6. Autoclave all clean-up materials before disposal in the biohazard waste container.
7. Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

**E. Small Spill of Material Outside of a BSC**

1. Alert laboratory employees.
2. Wearing gloves, safety glasses, and a lab coat, cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
3. Report the spill to the laboratory's PI, who will report the spill to the RO if a select agent or toxin is involved.
4. Pick up the towels and discard into a biohazard container. Pick up any pieces of broken glass with forceps and place in sharps container.
5. Re-wipe the spill area with disinfectant and thoroughly wash hands after glove removal.

**F. Large Spill of Biohazardous Material (>500 ml) Outside of a BSC**

1. Hold your breath and leave the room immediately.
2. Warn others to stay out of the spill area to prevent spread of contamination.
3. Post a sign stating: "DO NOT ENTER, BIOHAZARD SPILL, contact (name and phone #) for information."
4. Remove any contaminated clothing, ensuring that clothing is not pulled over the face, and put into a biohazard bag for later autoclaving.
5. Wash hands and exposed skin.
6. Notify the Principal Investigator, supervisor, and EH&S.
7. Put on protective clothing (lab coat, gloves and, if indicated, respirator, eye protection, shoe covers) and assemble clean-up materials.
8. Wait 30 minutes before re-entering the contaminated area to allow for dissipation of aerosols.
9. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
10. Collect and autoclave all treated material and discard in a biohazard container. Pick up any broken glass with forceps and place them into a sharps container.
11. Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of clean-up.

**G. Small Spill (<500 ml) of Recombinant DNA Materials**

1. Put on gloves and eye protection if you are not already wearing them.
2. Cover spilled material with an absorbent paper towel or Kimwipe. Once the absorbent material is in place over the spill, wet the material with a 10% solution of bleach or other appropriate disinfectant.
3. Let stand 15 minutes, wipe up and wash surface with appropriate disinfectant.
4. Wipe down all equipment and surfaces which may have been splashed.
5. Dispose of contaminated paper towels as infectious waste.

**H. Large Spill (>500 ml) of Recombinant DNA Materials in a BSC**

1. BSC must run during cleanup to contain aerosols and HEPA-filter exhaust air.
2. Don appropriate personal protective gear before initiating cleanup
3. Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is not acceptable.

4. If the spill is contained on a bench pad, remove the contaminated bench pad discard as infectious waste.
5. If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time then remove the contaminated towels and discard as infectious waste.
6. Wipe down the interior of the cabinet and any splatter on items within the cabinet with a disinfectant-soaked towel.
7. Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
8. Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
9. Place contaminated re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use.
10. If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required.
  - a. Ensure the drain valve under the cabinet is closed.
  - b. Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
  - c. Absorb spilled fluid-disinfectant from work surface with paper towels and discard in biohazard bag.
  - d. Prepare to empty drain pan. Place disinfectant solution in a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.
  - e. Open the drain valve and empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water and remove the flexible tubing. Manage contaminated materials as if they are infectious.
  - f. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands when gloves are removed.
  - g. Notify Principal Investigator, supervisor, and EH&S. Consult with EH&S to determine whether formaldehyde decontamination of the cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.
  - h. Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

#### **I. Large Spill (>500 ml) of Recombinant DNA Materials outside of a BSC**

1. If a spill of a biohazardous material occurs, outside the BSC, notify other individuals in the laboratory to evacuate.
2. Exit the laboratory to the hallway, closing the door behind you.
3. Remove any contaminated clothing (turn contaminated portion inward) and place it in an autoclave bag.
4. Wash all exposed skin.
5. Place signs on door(s) to the laboratory warning individuals who may want to enter that a spill occurred and access is denied.
6. Allow aerosols to settle for 30 minutes before re-entering the laboratory.
7. Notify the Principal Investigator, supervisor, and EH&S prior to proceeding with cleanup.
8. Assemble supplies (e.g., disinfectant, sharps containers, towels, tongs, autoclave bags) before entering the laboratory.
9. Don appropriate personal protective equipment (i.e. disposable gown, protective eyewear, gloves, shoe coverings and respiratory protection [if needed]).
10. Clean up spill with a suitable disinfectant as follows:
  - a. Surround spill area with disinfectant or diking material that is soaked in disinfectant.
  - b. Place paper towels soaked in a disinfectant over the entire spill area.
  - c. Allow 20-minute contact time with the disinfectant to ensure adequate germicidal action.



- d. Wipe down non-autoclavable materials with germicidal disinfectant.
- e. Place items designated as contaminated used sharps in an appropriate infectious waste sharps container. Place other disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
- f. Place contaminated re-usable items in biohazard bags or autoclavable pans with lids. Sterilize preferably by autoclaving, and then clean for re-use. Remove protective clothing used during cleanup then place in a biohazard bag for autoclaving.
- g. Wash hands when gloves are removed.

## **J. Spill of Recombinant DNA Materials in a Centrifuge**

A single centrifuge spill or release can lead to multiple infections in a laboratory. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. Therefore, whenever opening a centrifuge, it must be performed slowly.

### **Unsealed Buckets**

1. If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. If breakage is discovered after the machine has stopped, re-close the lid immediately and allow the unit to be at rest for 30 minutes.
2. Unplug centrifuge before initiating clean up.
3. Don strong, thick, rubber gloves, and other PPE before proceeding with clean up.
4. Flood centrifuge bowl with a germicidal disinfectant. Place paper towels soaked in a disinfectant over the entire spill area. Allow a 20 minutes contact time.
5. Use mechanical means (e.g., forceps) to remove broken tubes and glass fragments. Place them in a sharps container for autoclaving and disposal as infectious waste.
6. Remove buckets, trunnions, and rotor and place in disinfectant for 20 minutes or autoclave.
7. Unbroken, capped tubes may be placed in disinfectant and recovered after 20 minutes contact time or autoclaved.
8. Use mechanical means to remove remaining disinfectant soaked materials from centrifuge bowl and discard as infectious waste.
9. Place paper towels soaked in a disinfectant in the centrifuge bowl and allow it to soak overnight, wipe down again with disinfectant, wash with water and dry. Discard disinfectant soaked materials as infectious waste.
10. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands whenever gloves are removed.
11. Notify Principal Investigator, supervisor, and EH&S.

### **Sealed Buckets (Safety Cups)**

1. If breakage is suspected, remove the sealed bucket to a biological safety cabinet before opening.
2. If breakage occurred, replace the cap on the safety cup loosely and autoclave.
3. Notify Principal Investigator, supervisor, and EH&S.

## **K. Reporting Exposures**

In the event of an exposure to a biohazardous material:

1. Report to an Occupational Health Nurse or a primary healthcare provider.
2. Complete an Accident-Illness Report Form and submit to EH&S.
3. Work with the Principal Investigator, supervisor, and EH&S to report accident to the NIH Office of Biotechnology Activities as required by the *NIH Guidelines*.

## **X. TRANSFERS, PACKAGING, AND SHIPPING OF BIOLOGICAL MATERIALS**

### **A. Transfers**

The transferring, packing and shipping of select agents and toxins is HIGHLY regulated. No select agent or toxin shall be transferred, packed, or shipped without the express approval from the RO. Please see the appendix titled "Special Considerations for Select Agents and Toxins" for additional information.

For materials that are not Select Agents, each PI must develop procedures for transferring or shipping from the laboratory. The PI must ensure the following:

- Package, label, and transport biohazardous agents in compliance with all applicable local, federal, and international transportation and shipping regulations, including US Department of Transportation (DOT) regulations. Materials that are transported by airline carrier should also comply with packaging and shipping regulations set by the International Air Transport Association (IATA). Personnel who package, handle, and ship these agents (including import and export) are subject to all applicable training. Please refer to EHS [Form 406](#). The RO must be notified of all select agent transfers; internal or external (see section below for additional information).
- Required permits (e.g., granted by the US Public Health Service, USDA, DOT, US Department of Commerce and IATA) are obtained before biohazardous agents are prepared for transport. Standard operating procedures should be in place for import and export activities.
- Decontaminate contaminated or potentially contaminated materials before they are removed from the laboratory area.
- Avoid hand-carrying biohazardous agents when transferring them to other external facilities. If biohazardous agents are to be hand-carried on common carriers, all applicable packaging, transport, and training regulations should be followed.
- Develop and follow a protocol for intra-facility transfer (between laboratories on ASU campuses) of all biological and biohazardous agents. Contact EHS for assistance.

Packaging and shipping of biological materials must be completed in a way that ensures the contents will not leak and that the package will arrive in good condition.

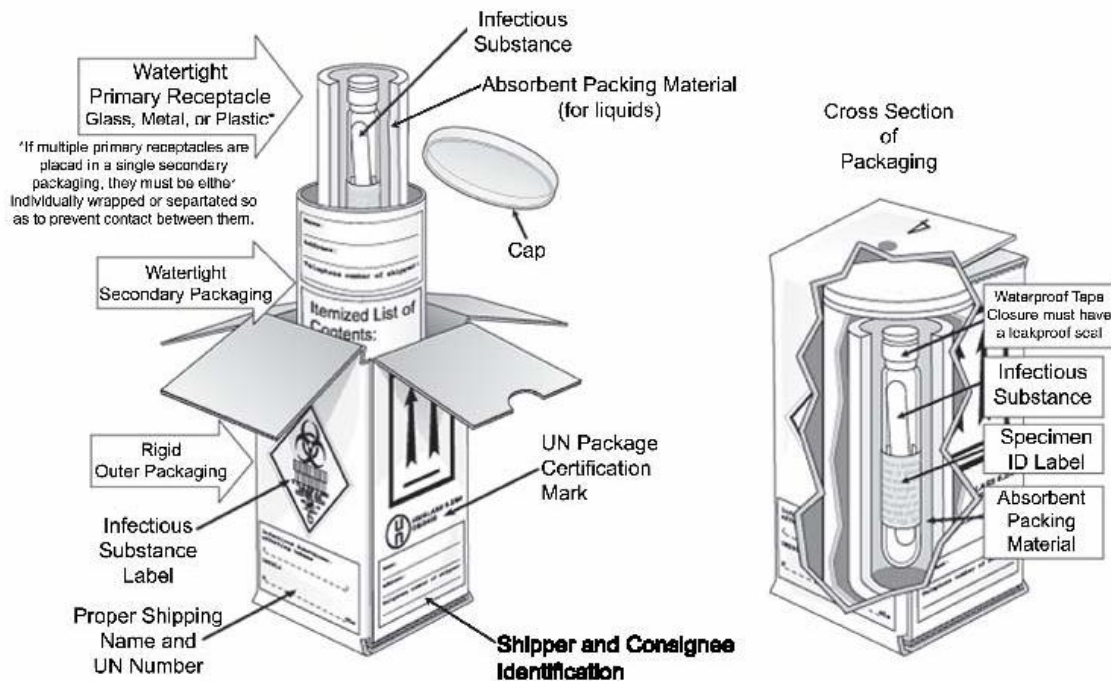
The definitions below apply to the packaging and shipping instructions that follow:

- **Etiologic agent** means a viable microorganism or its toxin which causes, or may cause, human disease.
- **Diagnostic specimen** means any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, etc., which is reasonably believed to contain an etiologic agent and is being shipped for purposes of diagnosis.
- **Biological product** means a biological prepared and manufactured in accordance with regulations that govern the manufacture of vaccines, reagents, etc.
- **Interstate shipping** means shipping across state lines within the continental United States. Intrastate shipping means shipping within the State of Arizona.

### **B. Packaging**

All biological materials including diagnostic specimens and biological products that may contain an etiologic/biohazardous agent must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions possible with ordinary handling and transportation (e.g., passage through cancellation machines, sorters, conveyors). Contents should not leak to the outside of the shipping container even if leakage of the primary container occurs.

Specific packaging requirements apply to materials that are known to contain, or reasonably believed to contain certain etiologic agents. For such materials the following procedures apply (See Figure 1. Source: *Biosafety in Microbiological and Biomedical Laboratories 5<sup>th</sup> Edition, Appendix C*).



**Figure 1**

**Volume not exceeding 50 milliliters (mL):**

1. Place material in a securely enclosed, watertight primary container (test tube, vial, etc.). Enclose this primary container in a secondary, durable, watertight container. Several primary containers may be enclosed in a single secondary container as long as the total volume of material in all the primary containers enclosed does not exceed 50 ml.
2. Place absorbent nonparticulate material (e.g., paper towels, **not** sawdust or vermiculite, etc.) in the spaces at the top, bottom, and sides between the primary and secondary containers. Use enough absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage.
3. Enclose each set of primary and secondary containers in an outer shipping container constructed of corrugated fiberboard, cardboard, wood or other material of equal strength. Do not use bags, envelopes, or similar materials.
4. If you package the material with dry ice, see the section below (Packaging with Dry Ice).

### **Volume greater than 50 ml:**

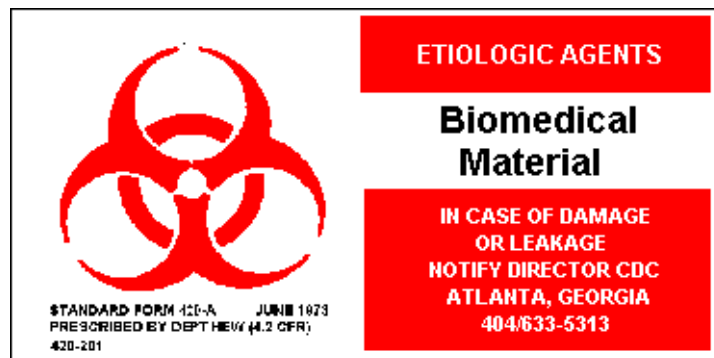
1. Follow requirements for lesser volumes outlined above.
2. Place shock absorbent material at the top, bottom, and sides between the secondary container and the outer shipping container. (This material should at least equal the amount of absorbent material placed between the primary and secondary containers).
3. Ensure single primary containers contain no more than 1000 ml of material; however, two or more primary containers (combined volumes not exceeding 1000 ml) may be placed in a single secondary container. The maximum amount of etiologic agent which may be enclosed within a single outer shipping container must not exceed 4000 ml.

### **C. Packaging with Dry Ice**

1. If used, place dry ice between the secondary and outside containers.
2. Place shock absorbent material so as to prevent the secondary container from becoming loose inside the outer container as the dry ice sublimates.
3. Use the DOT dry ice label. Guidelines for shipping with dry ice can be found at EHS [MazMat shipping](#).

### **D. Labeling**

The outer shipping container of all materials containing etiologic/biohazardous agents which are being shipped or transported must bear a special label, as illustrated in *Figure 2*. These labels are available from your laboratory supply vendor.



**Figure 2 – Example of a Etiological Agent Label**

### **E. Shipping and Transportation Methods and Requirements**

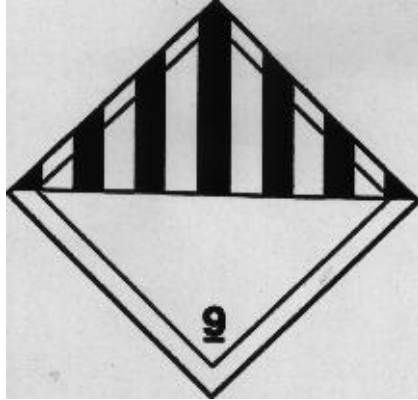
#### **Registered Mail or the Equivalent**

For a list of etiologic agents that use registered mail or an equivalent system which provides the sender with immediate notification of receipt refer to Appendix A of the CDC Guidelines on Additional Requirements for Facilities Transferring or Receiving Select Agents and Toxins, 49 CFR Part 72.

#### **Federal Express or UPS**

1. For Federal Express/UPS shipments, internationally **or** domestically, follow the International Air Transport Association (IATA) Dangerous Goods Regulations. (Receipt of shipment notice is not required since the shipment is traceable through the specific carrier.)

2. Apply appropriate labels to the outer shipping container for packages containing dry ice and/or biohazardous substances as shown in *Figures 3 and 4*, respectively.
3. Contact the specific carrier's dangerous goods agent prior to shipment for any additional packaging and labeling requirements.



**Figure 3**



**Figure 4**

### **Damaged Packages**

When evidence of leakage or any other damage to packages bearing an Etiological Agents/Biomedical Material label is discovered, the carrier must promptly isolate the package and notify the Director, Centers for Disease Control and Prevention (CDC), (404) 633-5313, 1600 Clifton Road NE, Atlanta, Georgia 30333.

### **Notice of Delivery**

In the event that a package sent from ASU is not received by the recipient within 5 days following the anticipated delivery of the package, the sender must notify the Director, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, Georgia 30333 or by telephone (404) 633-5313.

### **Importation/Exportation of Etiologic Agents**

Importation of biohazardous agents, etiologic agents, and vectors that may contain such agents is governed by federal regulation. In general, an importation permit is required for any infectious agent known to cause disease to humans. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts, and molds. In some instances, an agent which is suspected of causing human disease also requires a permit.

Importation permits are issued by the U.S. Public Health Service (USPHS) only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by U.S. Customs.

Instead of an importation permit, a Letter of Authorization may be issued by the Centers for Disease Control and Prevention after review of an "Application to Import an Etiological Agent." The letter is issued for materials that are judged to be noninfectious, but which might be construed to be infectious by U.S. Customs inspection personnel. Letters of Authorization may be issued for items such as formalin-fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine, cerebrospinal fluid, and other tissues or materials of human

origin when there is no evidence or indication that such materials contain an infectious agent. Letters of Authorization are in effect for two years and do not require a shipping label to be issued by CDC.

Importation permits and Letters of Authorization are issued by the Biosafety Branch, Office of Health and Safety, CDC, 1600 Clifton Road, Atlanta, Georgia 30333, after review of a completed application form. Application forms may be obtained by calling CDC at their FAX Information System. Dial 1-888-CDC-FAXX and enter document number 101000. CDC can also be contacted on the Internet at <http://www.cdc.gov/od/eaipp/> . Completed forms may be returned to CDC by mail or FAX at 404-639-2294. Application to CDC for the importation permit should be made 15 working days in advance of the shipment date to allow time for processing, issuance, and delivery of the permit and shipping labels to the permittee.

#### **F. Other Permits**

APHIS permits are required for importation or domestic shipping of infectious agents of livestock, poultry, and other animal diseases, and any materials that might contain these agents. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture-grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U.S. Applications for USDA/APHIS permits may be obtained by calling the USDA/APHIS at (301) 734-3277 or through the Internet at <http://www.aphis.usda.gov/permits/index.shtml>.

The importation or domestic transfer of plant pests is also regulated by the USDA. Such a permit is required for plant pests, plant biological agents, or any material that might contain them. Information may be obtained by calling (301) 734-3277 or through the Internet at [http://www.aphis.usda.gov/plant\\_health/permits/index.shtml](http://www.aphis.usda.gov/plant_health/permits/index.shtml)

USDA permits are required for certain live animals and all live bats. Call (800) 358-2104 for further information.

Export of infectious materials may require license from the Department of Commerce (DoC). Exporters of a wide variety of etiologic agents of human, plant, and animal diseases, including genetic material and products which might be used for culture of large amounts of agents will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the Internet at <https://bxa.ntis.gov/>.

## **XI. SECURITY**

Laboratory security is an integral part of an effective safety program. Follow these steps to ensure a secure working environment in your laboratory:

1. Keep laboratory doors closed and locked when unoccupied.
2. Keep stocks of organisms and hazardous chemicals locked when the laboratory is unoccupied.
3. Keep an accurate record of chemicals, stocks, cultures, project materials, growth media, and those items that support project activities.
4. Notify ASU police if materials are damaged or missing from laboratories.
5. Inspect all packages arriving into the laboratory.
6. When research is completed for the day, ensure that chemicals and biological materials have been stored properly and securely.
7. Decontaminate materials and work surfaces after completing work and at least daily.
8. Turn off equipment, flames, steam supply, and electrical appliances after completing work.
9. Ask strangers (someone you do not recognize as a co-worker or support staff person) to exit the room if they are not authorized to be there.
10. Discuss other security-specific requirements with your supervisor and colleagues.

Security procedures for laboratories with select agents and toxins can be found in the appendix titled "Special Considerations for Select Agents and Toxins."

## **XII. COMPLIANCE**

ASU Environmental Health and Safety (EH&S) will conduct regular inspections, at least annually, of each laboratory to ensure compliance with the procedures and protocols of this manual. Inspection reports will document violations and be directed to the PI. The results of these inspections will be documented and kept on file with EH&S. Any significant problems will be reported to the IBC.



### **XIII. RECORDKEEPING**

At a minimum, the PI must maintain the following records and be prepared to present these at the annual laboratory inspection:

- An accurate, current list of each biological agent or toxin stored in that room;
- A Health Hazard Assessment for each biological agent or toxin stored in that room;
- A current [Responsible Information Party Sheet](#);
- Training Documentation Forms;
- Safety, security, and emergency response plans; and
- Safety and security incident reports.

#### **XIV. PROGRAM EVALUATION**

The review of the elements as noted in the Compliance and Recordkeeping sections of this document will constitute an evaluation of the ASU Biosafety and Biosecurity Program.

**Table 1 - Disinfectant Activity**

Disinfectants	Practical Requirements					Inactivates						
	Type	Category	Use Dilution	Contact Time (min) Lipovirus:	Contact Time (min) Broad Spectrum	Temperature (C°)	Relative Humidity (%)	Vegetative Bacteria	Lipoviruses	Nonlipid Viruses	Mycobacteria	Bacterial Spores
Liquid	Quat. Ammon. Cpds	0.1%-2.0%	10	NE				+	+			
	Phenolic Cpds	1.0%-5.0%	10	NE				+	+	B		
	Chlorine Cpds	500 ppm*	10	30				+	+	+	+	+
	Iodophor	25-1600 ppm*	10	30				+	+	+		
	Alcohol, Ethyl	70%-85%	10	30				+	+	B		
	Alcohol, Isopropyl	70%-85%	10	30				+	+	B		
	Formaldehyde	0.2%-8.0%	10	30				+	+	+	+	+
	Glutaraldehyde	2%	10	30				+	+	+	+	+
Gas	Ethylene Oxide	8-23g/ft <sup>3</sup>	60	60	37	30		+	+	+	+	+
	Paraformaldehyde	0.3 g/ft <sup>3</sup>	60	60	>23	60		+	+	+	+	+

NE=not effective

B=Variable results dependent on virus

\*=Available halogen (1:100)

**Table 2 - Disinfectant Characteristics**

Disinfectants		Important Characteristics										
Type	Category	Effective Shelf Life > 1 week (A)	Corrosive	Flammable	Explosion Potential	Residue	Inactivated by Organic Matter	Compatible for Optics (D)	Skin Irritant	Eye Irritant	Respiratory Irritant	Toxic (E)
Liquid	Quat. Ammon. Cpds	+					+	+	+	+		+
	Phenolic Cpds	+	+			+			+	+		+
	Chlorine Cpds		+			+	+		+	+	+	+
	Iodophor	+	+			+	+		+	+		+
	Alcohol, Ethyl	+		+								+
	Alcohol, Isopropyl	+		+								+
	Formaldehyde	+				+			+	+	+	+
	Glutaraldehyde	+				+			+	+	+	+
Gas	Ethylene Oxide	N/A		+(B)	+(B)			+	+	+	+	+
	Paraformaldehyde	N/A		+(C)	+(C)			+	+	+	+	+

N/A=not applicable (A)=Protected from light and air (B)=Neither flammable nor explosive in 90% CO<sub>2</sub> or fluorinated hydrocarbon, the usual form (C)=At concentrations of 7%-73% by volume in air, solid exposure to open flame (D)=Usually compatible, but consider interferences from residues and effects on associated materials such as mounting (E)=By skin or mouth, or both. Refer to manufacturer's literature and the MSDS.

**Table 3 - Disinfectant Applications**

Disinfectants		Important Characteristics										
Type	Category	Work Surface	Dirty Glassware	Large Area	Air Handling	Portable Equip. Surface Decon	Port-able Equip. Penetrating Decon	Fixed Equip. Surface Decon	Fixed Equip. Penetrating Decon	Optical & Electronic Inst.	Liquid & Discard	Book, Paper
Liquid	Quat. Ammon. Cpds	+	+			+		+				
	Phenolic Cpds	+	+			+		+				
	Chlorine Cpds	+	+			+		+			+	
	Iodophor	+	+			+		+				
	Alcohol, Ethyl	+	+			+		+				
	Alcohol, Isopropyl	+	+			+		+				
	Formaldehyde	+	+			+		+				
	Glutaraldehyde	+	+			+		+				
Gas	Ethylene Oxide					+	+			+		+
	Parafomaldehyde			+	+	+	+		+	+		

## Appendix A: Definitions

**Biohazardous Agent:** Any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsia, protozoa or prions) or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing:

- Death, disease, or other biological malfunction in a human, animal, plant, or another living organism;
- Deterioration of food, water, equipment, supplies, or material of any kind; or
- Deleterious alteration of the environment.

### **Recombinant DNA:**

- 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
- Molecules that result from the replication of those described above, and
- Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide.

**Centers for Disease Control and Prevention (CDC):** The Centers for Disease Control and Prevention of the United States Department of Health and Human Services

**Etiologic Agent:** A viable microorganism or its toxin that causes, or may cause, human disease

**Overlap agent or toxin:** Any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa) or toxin that poses a risk to both human and animal health.

**Responsible Official:** The individual designated by an institution to act on its behalf. This individual must have the authority and control to ensure compliance with the regulations

**Toxin:** The toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes:

- Any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or
- Any poisonous isomer or biological product, homolog or derivative of such a substance.

## Appendix B: Lab-Specific Biosafety Manual Template

### Arizona State University Lab-Specific Biosafety Manual

***Template***

*[Your Name]*

*[Your Title]*

*[Arizona State University, Department Name]*

*[Building Name, Room Number]*

Tempe, AZ

*[Preparation Date]*

## **Instructions to Principal Investigators (PI) for completing this template for Lab-Specific Biosafety Manual**

Before completing this template for the use of select agents and toxins in your laboratory, please review the ASU Biosafety Manual and its appendices as well as the [CDC Guidelines for Working with Toxins of Biological Origin](#).

In addition, each PI must submit to EH&S the following:

- A completed [Responsible Party Information Sheet](#);
- An annual chemical and biological inventory; and
- A Health Hazard Assessment for each biological agent and toxin stored in the laboratory. A copy of this Health Hazard Assessment should be placed in Appendix B of this document.

Complete all highlighted sections with the laboratory policies and procedures specific to the biohazardous agent(s) in your laboratory. If you have any questions on completing these procedures, please contact the EH&S, (480) 965-1823, for additional assistance.



## SIGNATURE and ACKNOWLEDGEMENT PAGE

### Authorization [This section is based upon your specific laboratory setup]

All members of the [*Principal Investigator's name*] Laboratory who have signed the list below are approved for entry into Room [XXXX] while work with biohazardous agents is in progress.

Anyone (including any workers not in the PI's Lab) who uses Room [XXXX] lab must sign below.

### Acknowledgement

We, the undersigned, understand that [*the agents used in Room XXXX*] handled in Room [XXXX] of [*Building Name*] may be hazardous to humans. Furthermore, we have read and understood this manual and have attended ASU Laboratory Safety Training, ASU Bloodborne Pathogen Training, and Biosafety Training specific to the agent used in this laboratory prior to handling samples in Room [XXXX].

Name	Signature	Date	Agent Vaccination* (Yes/No/ Declined/NA)

---

\*Agent vaccination in table should be specified, as appropriate to potential exposure in the laboratory: HBV, Vaccinia, Influenza, etc.

## **PURPOSE**

This document, in addition to the ASU Biosafety Manual, provides a comprehensive information source for all matters covering the use of biohazardous agents handled in this laboratory [Room Address of Principal Investigator]. Specifically, it describes the procedures to be used to ensure a safe working environment while handling biohazardous agents. This manual will be reviewed annually by the Principal Investigator for changes or corrections to ensure that it is accurate.

*[The information in the following two sections is an example of the type of specific description of your operations and activities that you should include in your manual. You should discuss detailed procedures and the actual precautions to be taken by those who actually work with the biohazardous agents found in your laboratory.]*

## **BACKGROUND**

Description of work to be performed and biohazardous agents to be used (as shown in the following example):

Biological Hazards under Routine Use [insert a brief summary of the hazards]

Biological Hazards as result of a spill or other accident [insert a brief summary of the hazards]

Procedures involving the use of Biological Hazards [insert a step-by-step procedure for this work]

## **DESCRIPTION OF LABORATORIES**

[Provide a summary of required project equipment and a floor plan showing its location]. Laboratory(s) is/are located on the [xxx floor] of [Building Name] building within Arizona State University. All biohazardous agents manipulations occur(s) in Room(s) [XXXX].

Floor diagrams are displayed as Appendix A at the end of this manual. Ensure that the location of the sinks, eyewash, safety shower, spill kits, fire extinguishers, exits, MSDSs, and phones are included on the diagram.

**NOTE: PLEASE EDIT THE FOLLOWING POLICES AND PROCEDURES TO FIT YOUR LAB OPERATIONS. ENSURE THAT ALL ITEMS FROM THE ASU SAFETY BIOSAFETY MANUAL ARE INCORPORATED INTO THIS DOCUMENT.**

## **GENERAL LAB RULES**

Work with biohazardous agents will be performed in [Building Name, Room XXXX].

1. Laboratory employees must immediately notify the laboratory supervisor or PI in case of an accident, injury, illness, spill or exposure associated with laboratory activities.
2. Access to this area will be limited to persons meeting the following qualifications:
  - Have attended all applicable training sessions; and
  - Have read and signed this manual.
3. No eating, drinking, smoking, handling contact lenses, or applying cosmetics in [Room XXXX or Room YYYY] at any time. Persons who wear contact lenses should also wear goggles or a face shield while working with biohazardous agents. If laboratory employees elect to wear respirators, all aspects of ASU's Respiratory Protection Program must be followed. All fit testing and training documentation should be included in Appendix C.

4. Food, medications, or cosmetics should not be brought into the laboratory for storage or later use. Food is stored outside the work area in cabinets or refrigerators designated specifically for that purpose and labeled accordingly.
5. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Wash hands:
  - after handling biohazardous materials
  - after removing gloves, and
  - before leaving the laboratory.
8. Razor blades, scalpels, and hypodermic needles (“sharps”) should be used only when absolutely needed in [Room XXXX].
9. Work surfaces are decontaminated at least once a day and after any spill of biohazardous agents with [specify the appropriate disinfectant (refer to the ASU Biosafety Manual)].
10. All cultures, stocks, and other regulated biological wastes are decontaminated before disposal by [specify an approved decontamination method, such as autoclaving].
11. Plastic-ware should be substituted for glassware whenever possible.
12. When biohazards are in use, the room should be posted to indicate “Biohazards in Use - Authorized Personnel Only.” Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while biohazards are in use.
13. All high-risk operations should be conducted with at least two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

#### **CONTAINMENT REQUIREMENTS**

1. Preparation of biohazardous stock solutions and manipulations of high concentrations or large volumes of biohazard agents should be conducted in a biological safety cabinet or equivalent containment system approved by a licensed outside contractor.
2. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by a licensed outside contractor. HEPA and/or charcoal filtration of the exhaust air may be required.
3. The user should verify inward airflow of the fume hood or biological safety cabinet before initiating work.
4. All work should be done within the operationally effective zone of the fume hood or biological safety cabinet.
5. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Biohazardous agents and toxins should be transported only in leak-proof and spill-proof secondary containers.
6. Contaminated and potentially contaminated protective clothing and equipment should be

decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible or practical, materials (e.g., used gloves) should be disposed of as biological or toxic waste. Materials contaminated with infectious agents as well as toxins should also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.

7. The interior of the hood, glove box, or cabinet should be decontaminated periodically (e.g. at the end of a series of related experiments). Until decontaminated, the hood, box, or cabinet should be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.
8. When vacuum lines are used with systems containing toxins, they should be protected with a HEPA filter to prevent entry of toxins into the lines. Sink drains should be similarly protected when water aspirators are used.

### **PROPER USE OF BIOLOGICAL SAFETY CABINETS**

**[NOTE: PLEASE REFER TO THE SECTION ON BIOLOGICAL SAFETY CABINETS IN THE ASU BIOSAFETY MANUAL FOR ADDITIONAL INFORMATION.]**

Biological safety cabinets (BSC) must be certified prior to use. A qualified outside contractor must certify each BSC annually. Check the certification sticker on the front of the unit to verify your C's condition. The latest certification can be found in Appendix C.

To assure sterility inside the BSC and establish proper airflow for containment, the blower should be turned on at least ten minutes before infectious materials are put in the BSC.

The BSC airflow ("magnahelic") gauge should be checked to assure proper operation of the cabinet before placing any materials into it. (Reading is equal to approximately 0.5 inches.) Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity.

After use, wipe inner surfaces (especially the pan) with a solution of **[The disinfectant chosen as effective for the agent you are using (refer to Table 1 in the ASU Biosafety Manual)]** and allow to dry. Always keep a bottle of the disinfectant in the cabinet for decontaminating or in case of a spill.

#### **NEVER place anything over the front or rear grill of a biosafety cabinet.**

Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet. It also allows non-sterile air from the room to blow into the biosafety cabinet over the experiments.

Materials should be placed in the cabinet so as not to block airflow into the rear grill. Leave a few inches for air to flow around objects. Any disruption of the air flow in the cabinet decreases its effectiveness.

Remember: **"A biosafety cabinet is only as safe as the person using it."**

Before manipulating infectious materials, make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.

Work should be performed in the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated). However, biohazardous agents should not be placed directly adjacent to or directly on the intake grills.

After manipulating biohazardous agents, make sure all containers are tightly closed before removing them. Wipe down the surface of all equipment used in manipulations (mechanical pipettes, etc.) with disinfectant before removing from the cabinet. All waste and disposable items should be left in the cabinet until properly decontaminated or contained.

After the cabinet has been emptied, wipe exposed surfaces including the front grill and splash area with disinfectant. Allow the blower to run for a minimum of ten minutes to purge any potentially infectious aerosols from inside the cabinet before shutting off the blower.

**NOTE:** Class IIA cabinets recirculate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents, including ethanol and isopropanol, should be kept to a minimum or done elsewhere. Dangerously high levels of volatile fumes can accumulate inside the cabinet and pose a threat of fire or explosion.

## **DECONTAMINATION**

[NOTE: PLEASE REFER TO THE SECTION ON EMERGENCY RESPONSE IN THE **BIOSAFETY MANUAL FOR ADDITIONAL INFORMATION.**]

The decontaminant of choice is [xxxx]. (Please see Table 1 in the ASU Biosafety Manual for disinfectant selections)].

Pans and splash guards (inside and out) should be cleaned after every use.

All material and equipment contaminated with or containing potentially biohazardous agents should be decontaminated:

- Upon completion of procedures involving the use of biohazardous agents;
- In the event of spills of such materials;
- At least daily; and
- Before being washed, stored, or discarded.

Pipettes and other shared small equipment should be wiped with appropriate disinfectant after use and **before** removing them from the cabinet.

## **BIOLOGICAL WASTE DISPOSAL**

Wastes associated with biohazardous agents must be disposed of in special ways because they may have been contaminated with infectious organisms or toxic agents. These potentially infectious items include all sharps, e.g. glass implements, needles, syringes, blades, etc. coming from facilities using infectious materials; and biologically cultured stocks and plates, human blood or tissues.

For disposal of these wastes, the personnel must:

- Sterilize or disinfect waste materials associated with viral, bacterial or other agents infectious to humans by autoclaving or disinfectant equivalent to 1:10 bleach solution;
- Disposed of or destroyed toxins per the Lab-Specific Biosafety Manual;
- Place all biohazardous wastes, **except for sharps**, directly into the red bag-lined medical waste boxes. The procedures for biohazardous waste handling are described in the [ASU Biological Hazardous Waste Management Compliance Guidelines](#).

## **PERSONAL PROTECTIVE EQUIPMENT**

1. When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment)

should be worn so that hands and arms are completely covered.

2. Eye protection should be worn when handling a biohazardous agent or toxin, infectious organism, or chemicals (e.g. ethanol or other disinfectant).
3. Other protective equipment may be required, depending on the characteristics of the biohazard or toxin and the containment system. [INSERT ANY ADDITIONAL REQUIRED PPE HERE]
4. When handling dry forms of toxins that are electrostatic:
  - Do not wear gloves (such as latex) that help to generate static electricity; and
  - Use glove bag within a hood or biological safety cabinet, a glove box, or a Class III biological safety cabinet.
5. When handling biohazardous agents that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the biohazard or toxin. GLOVES WORN IN THE LABORATORY WILL BE XXX.
6. Consider the chemical characteristics and potential routes of exposure of biohazardous agents, diluent, and disinfectant when selecting gloves and other protective clothing.
7. If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

### **MANAGEMENT OF SPILLS**

[NOTE: PLEASE REFER TO THE SECTION ON BIOLOGICAL MATERIAL SPILLS IN THE ASU BIOSAFETY MANUAL TO COMPLETE THIS SECTION.]

Inside of a containment device such as biosafety cabinet:

- Alert the other laboratory employees;
- **Leave the cabinet turned on;**
- While wearing gloves, lab coat, and safety glasses, spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes;
- Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill;
- Autoclave all clean-up materials before disposal in the biohazard waste container. Wash hands and any exposed surfaces thoroughly after the clean-up procedure; and
- Report the spill to the laboratory's PI.

Small spills of infectious material outside of a containment device:

**All spills must be reported to [the Principal Investigator].**

**All spills of any nature must be described in your laboratory notebook.** The description must include:

- The name of the biohazardous agent and any identifying information (e.g., strain or other characterization information);
- An estimate of the quantity released;
- The time and duration of the release;

- The environment into which the release occurred (e.g., in building or outside of building, waste system);
- The location [building, room] from which the release occurred;
- The number of individuals potentially exposed at the facility;
- Actions taken to respond to the release; and
- Hazards posed by the release.

## **MANAGEMENT OF ACCIDENTAL EXPOSURES**

In the event of an exposure to a biohazardous agent:

### **Intact skin**

- Remove contaminated clothing;
- Vigorously wash contaminated skin for 1 minute with soap and water;
- Inform the laboratory's PI and seek medical attention at the ASU Campus Health Services.

### **Broken, cut, or damaged skin or puncture wound**

- Remove contaminated clothing;
- Vigorously wash contaminated skin for 5 minutes with soap and water; and
- Inform the laboratory's PI and seek medical attention at the ASU Campus Health Services.

### **Eye**

- Immediately flush eyes for at least 15 minutes with water, using an eyewash;
- Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly; and
- Inform the laboratory's PI and seek medical attention at the ASU Campus Health Services.

### **Ingestion or Inhalation**

- Inform the laboratory's PI and seek medical attention at the ASU Campus Health Services; and
- Do not induce vomiting unless advised to do so by a health care provider.

## **MEDICAL SURVEILLANCE**

Specific biohazards and toxins will be evaluated by [PI's name] for appropriate medical surveillance procedures. [Insert any applicable procedures].

## **TRAINING**

[NOTE: PLEASE REFER TO THE SECTION TRAINING IN THE ASU BIOSAFETY MANUAL TO COMPLETE THIS SECTION.]

Laboratory personnel shall demonstrate the following:

- Understand the hazards associated with [the biohazardous agent];
- Understand the required practices and procedures for working with [the biohazardous agent];
- Know the necessary precautions to prevent exposures; and
- Know the safety and security policies and procedures in place for reporting and investigating unintentional injuries, spills, incidents (e.g., unauthorized personnel in restricted areas, missing biologic agents or toxins, and unusual or threatening phone calls), or breaches in security measures.

This document will provide the basis of training.

[The PI] will provide information and training at the time of an individual's initial assignment to a work area where biohazardous agents are present and prior to assignments involving new potential exposure situations. [The PI] will provide refresher training at least annually and when there are any changes in

processes or procedures. [Current training records and an outline of the training program can be found in Appendix C.]

## **RECEIVING BIOLOGICAL AGENTS**

[NOTE: PLEASE REFER TO THE SECTION ON RECEIVING BIOLOGICAL AGENTS IN THE ASU BIOSAFETY MANUAL TO COMPLETE THIS SECTION.]

Biohazardous agents will be received into the laboratory by [insert procedure here].

## **EMERGENCY PHONE NUMBERS AND PROCEDURES**

Emergency Phone Numbers [insert any additional contacts here]

Fire and Medical Emergencies	911
Police	911
Principal Investigator's Home Phone	xxx-xxxx
Campus Health	965-3346
Hospital Emergency Room	xxx-xxxx
ASU Department of Public Safety	965-3456
Department of Environmental Health & Safety	965-1823

## **Emergency Procedures**

[NOTE: PLEASE REFER TO THE SECTION ON EMERGENCY RESPONSE PROCEDURES IN THE ASU BIOSAFETY MANUAL TO COMPLETE THIS SECTION.]

Principal Investigators must be aware of the provisions for emergency procedures and preparedness. Emergency procedures and preparedness must be incorporated into specific biosafety standard operating procedures (SOP) for each biohazardous agent used in the laboratory. The Lab-Specific Biosafety Manual must address the following:

- The hazards associated with the use of the biohazardous agent;
- Personal protective equipment and emergency equipment;
- Site security and control;
- Emergency recognition and prevention;
- Any hazards associated with response actions that could lead to a spread of a biohazardous agent;
- Roles of the laboratory faculty and staff in the event of an emergency;
- Decontamination;
- Emergency alerting and response procedures;
- Emergency medical treatment and first aid;
- Safe distances and places of refuge;
- Evacuation routes and procedures;
- Any additional special procedures needed to address the hazards of the specific agents; and
- Reevaluate the ER Plan at least annually. Train employees and conduct exercises of the emergency response plan at least annually.



## **SPECIMEN TRANSPORT**

Biohazardous agents will be transported in the laboratory within a secondary container.

## VALIDATION AND HISTORY FOR BIOSAFETY MANUAL

### Principal Investigator's Certification

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating policy for the laboratories #[XXXX] and [YYYY] located in [Location] research building.

[Principal Investigator's Name]

[Principal Investigator's Title]

Signature \_\_\_\_\_ Annual Review Date \_\_\_\_\_

### Approvals

Department of Environmental Health & Safety

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Department Chair

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Institutional Biosafety Committee Chair

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

### History of Manual's Creation

Date Created:

Author:

Reference Source(s): [Insert P.I. NAME AND ANY APPLICABLE DOCUMENTS]

## **Appendix B-1: Floor Diagram**

Include the location of sinks, eyewash, safety shower, spill kits, fire extinguishers, exits, MSDS and phones.

## Appendix B-2: Health Hazard Assessment Worksheet

### Instructions to Principal Investigators (PI) Conducting a Health Hazard Assessment for biological or select agents at ASU Laboratories

Before completing this Health Hazard Assessment (HHA), please review the ASU Biosafety Manual. In addition, it may be helpful to refer to the [Public Health Agency of Canada's MSDS](#) website.

Complete all sections accurately of the attached worksheet. This information must be included in the Lab-Specific Biosafety Manual. If you have any questions on completing these procedures, please contact Environmental Health & Safety, (480) 965-1823, for additional assistance.

The Principal Investigator (PI) is responsible for initially assessing health hazards in order to set the biosafety level for the work to be done. This must be done in close collaboration with Environmental Health & Safety and Animal Care (if applicable) to ensure compliance with established guidelines and regulations. The PI should be aware of the following factors prior to conducting the HHA:

1. Biological, chemical, and physical hazards in the laboratory
2. Principles of biological, chemical, and physical safety
3. Method of transmission of various infectious agents
  - Direct versus Indirect Contact
    - Direct contact (i.e., between humans, between humans and animals)
    - Indirect contact through common environmental surfaces (e.g., lab benches, equipment)
  - Airborne versus Vehicle
    - Airborne (i.e., inhalation of particles into the respiratory system)
    - Vehicle (i.e., sharps)
  - Vectors
    - Vector-borne (e.g., insects, mites, fleas)
4. Route of Infection
  - Ingestion
  - Inhalation
  - Inoculation
  - Penetration of abraded skin or mucous membrane through skin (e.g., cuts, abrasions)
  - Bites (animals or insect) Contact with mucous membranes of eyes, nose, and mouth
5. Biological Agent
  - Pathogenicity (severity of ability to cause infection). The virulence of microorganisms varies greatly among types and strains. [Some microbes are highly pathogenic, even in healthy adults, while others are opportunistic and only able to infect hosts with a lowered immunity.]
  - Infectious or intoxicating dose (i.e., the number of microorganisms required to initiate an infection).
  - Effect of route of exposure (infectious dose and intoxicating dose vary with route of exposure).
  - Virulence (primary or secondary communicability) of microorganisms varies greatly among types and strains.
  - Host susceptibility or resistance (genetic composition, age, gender, race). Conditions that alter host defenses at body surfaces or impair the functioning of the immune system may put a worker at increased risk.
  - Biological modifiers to resistance (preexisting condition, secondary infection, pregnancy, stress, immunosuppression, vaccination).
  - Viability. If the agent is not viable and able to replicate, possibility of infection does not exist.
  - Sensitization reactions (allergen, acute hypersensitivity).
  - Incidence of laboratory infections.
  - Treatment (success rate considering various routes of exposure and doses, antibiotic resistance and susceptibility).
  - Environmental impact (survival and dissemination in the environment, remediation, public health concerns, impact on humans, flora and fauna).

- Unique animal hazards:
    - Species ectoparasite vectors (fleas, ticks, lice).
    - Species specific in apparent infections (SIV, Monkey B Virus, TB, Hepatitis).
    - Agent excretion in urine, feces, saliva, blood (offspring) transmission.
    - Escape from facility.
6. Environmental Factors
    - Ventilation and laboratory design (directional air, pressure gradients, air breaks, separation of laboratories from offices, interlocking autoclave and airlock doors).
    - Laboratory procedures (use of sharps, mouth pipetting, minimization of creating aerosols).
    - Containment equipment (Class II and III biosafety cabinets, sealed centrifuges cups and rotors).
    - Personal protective equipment (gloves, goggles, respirators, aprons, gowns).
    - Training (handling, containment, destruction and transporting pathogens, emergency evacuation, spill procedures).
    - Laboratory sanitation (decontamination, housekeeping, disinfection, routine cleaning, pest and rodent control).
  7. Effects of aerosol-generation in the lab
    - How aerosols act as modes of transmission for infectious agents and chemical vapors?
    - What procedures are likely to cause aerosols?
    - What methods can be used to reduce or contain aerosols?
  8. Essential safety features necessary to lab operations
    - Air handling system.
    - Safety equipment.
    - Adequacy and limitations for decontaminating waste.
  9. Type of medical surveillance needed based on each employee's job duties. A medical surveillance program ensures that safeguards decided upon in fact produce the expected health outcomes. It may include serum banking, monitoring employee health status, and participating in post-exposure management.
  10. How the facility, equipment, personnel, procedures, and hazardous materials must be integrated to create a safe work environment.
  11. Local, state, and federal regulations under which the lab operates.
  12. Concentration. The number of infectious organisms per unit volume is important to know because an increase in the working volume of high-titered microorganisms increases the risk of infection.
  13. Origin. This refers to the geographic location, natural host, or the nature of the source.
  14. Availability of data from animal studies.
  15. Availability of an effective prophylaxis or therapeutic intervention.
  16. Experience and skill level of "at-risk" personnel.

In the United States, the most current classification of biological agents is found in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (*NIH Guidelines*), May 1999. The *NIH Guidelines* classify biological agents (see Table below) known to infect humans as well as select animal agents that may pose theoretical risk if inoculated into humans. Risk Groups established by the NIH correspond to the Biosafety Levels established by the Centers for Disease Control (CDC).

## **Transmission of Disease**

It is important to understand how an infectious disease is transmitted before conducting any work with an agent of the disease. This should be noted in the PI's Health Hazard Assessment. The interactions among the biological agent, human, and environment do not always result in disease and/or death. For an infection or illness to occur in a human, the following conditions must be met:

- The biological agent must be capable of causing disease;
- There must be a reservoir of sufficient number for the agent to live and reproduce;
- The agent must be able to escape the reservoir;
- The agent must be able to move through the environment (e.g., airborne, contact, vectors);
- There must be a means of transmission into the host (e.g., broken skin, inhalation, blood transfer, mucous membrane); and
- The human must be susceptible to the agent.

Every biosafety control strategy is intended to break one or more of the links in the transmission chain. One of the basic principles of biosafety is performing a Health Hazard Assessment (described in Administrative Controls), which involves understanding how an agent is transmitted to humans.

### **Direct Transmission**

Direct transmission from host to host occurs anytime an infected host transmits the disease to a susceptible host by any number of means. The most frequent portal of entry into the human body occur at sites where mucous membranes meet with the skin, including respiratory (upper and lower airways), gastrointestinal (primarily mouth), genital, and urinary tracts. Abnormal areas of mucous membranes and skin (e.g., cuts, burns, and other injuries) are also frequent sites of direct transmission.

### **Indirect Transmission**

Indirect host-to-host transmission occurs by either living or inanimate means. Living agents transmitting infection are called vectors; they are generally arthropods (e.g., insects, mites, fleas) or vertebrates (e.g., dogs, rodents). Arthropods may not actually be hosts for the disease but rather carriers. Indirect transmission by a living agent may occur from patient to patient on the hands of health care workers.

Inanimate objects such as bedding, toys, books, or surgical instruments may also transmit disease. These inanimate objects are collectively called fomites. If the inanimate object is food, water, or air, it is referred to as a disease vehicle.

**Steps for completing the Health Hazard Assessment:**

1. Identify the agents to be used;
2. Gather and evaluate existing data on the agents looking at the factors listed above;
3. Evaluate ways the agents can escape containment;
4. Determine the training required of personnel handling the agent;
5. Determine proper lab procedures;
6. Determine personal protective equipment required;
7. Determine safety equipment and containment needs;
8. Assess the capability of each facility to contain the material during normal and emergency situations
9. Determine potential exposure limits;
10. Consider whether the acceptance of risk is voluntary or imposed;
11. Recognize factors that give the illusion of no risk (e.g., unknown history of the agent, lack of toxicity testing);
12. Fill out the Environmental Health & Safety Health Hazard Assessment Worksheet (attached below); and
13. Include your completed Health Hazard Assessment Worksheet in your Lab-Specific Biosafety Manual.

(There are several systems for classifying human and animal pathogens. All systems are based upon the pathogenicity of the organism, mode of transmission, host range, availability of effective preventative measures and/or effective treatment.)

<b>Principal Investigator:</b>		<b>Phone No:</b>	
<b>Department:</b>		<b>Fax No:</b>	
<b>Building, Room, Mail Code:</b>		<b>Email:</b>	

Please fully complete each section.

### 1. Agent Identification

**Name (genus/species):**

**Type of Agent (check all that apply)**

- Bacterium:   
Parasite:   
Fungus:   
Rickettsia:   
Prion:   
Toxin derived from living organism:   
Virus:   
Recombinant DNA:

For recombinant DNA, the following questions must be answered:

- 1) Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?  
No:  Yes:  If yes explain:
- 2) Does the modification have the potential to alter the host range or cell tropism of the virus?  
No:  Yes:  If yes explain:
- 3) Does the modification have the potential to increase the replication capacity of the virus?  
No:  Yes:  If yes explain:
- 4) Does the inserted gene encode a known oncogene?  
No:  Yes:  If yes explain:
- 5) Does the inserted gene have the potential for altering the cell cycle?  
No:  Yes:  If yes explain:
- 6) What is the probability of generating replication-competent viruses?  
No:  Yes:  If yes explain:

### 2. Health Hazards

**Disease(s) caused by agent:**

**Pathogenicity:**

**Species:**

Potential pathogen:



Pathogenic:

Highly pathogenic:

Opportunistic pathogen:

Other:

**Epidemiology:**

**Host Range (check all that apply):**

Humans:

Animals:  Specify:

Plants:  Specify:

**Concentration (number of organisms per unit volume):**

**Infectious Dose:**

Known:

Unknown:

**Mode of Transmission (check all that apply):**

Direct Contact:

Human/human:

Human/animal:

Indirect Contact:

Vector-borne:

Inanimate objects:

Food, water, air:

Airborne:

Vehicle:

Vector:

Route(s) of Entry: (check all that apply)

Ingestion:

Inhalation:

Penetration through skin:

Contact with mucous membranes of eyes, nose, and mouth:

**Incubation Period:**

**Dissemination:**

Reservoir:

Zoonosis:

No:

Yes:  Name:

Vectors:

None:

Yes:  Name:

**Viability:**

Drug Susceptibility:

Drug Resistance:

Disinfectant Susceptibility:

Physical Inactivation:

Survival Outside Host:

**Medical:**

Surveillance/ known symptoms of infection:

First Aid/Treatment:

Immunization:

Prophylaxis:

**Laboratory Hazards:**

Laboratory-Acquired Infections (epidemiology of lab-acquired infections):

Sources/Specimens:

Primary Hazards:

Flammable:  Reactive:  Carcinogenic:  Toxic:  Corrosive:  Oxidizer:

Special Hazards:

**Recommended Precautions:**

Containment Requirements:

Personal Protective Equipment:

Staff Trained and Experienced with Agent:

Other Precautions:

**Handling Information:**

Spills:

Disposal:

Storage:

**Miscellaneous Information:**

**Congratulations! You now have a material safety data sheet for your biological agent.**

Principal Investigator's Signature \_\_\_\_\_

Title: \_\_\_\_\_ Date: \_\_\_\_\_

Updated: \_\_\_\_\_

**Please forward a copy of this form to Environmental Health & Safety, Mail Code 5412**

### **Appendix B-3: IBC Application Documentation**

Insert the completed and approved [EHS 112 form](#)(s) at this appendix.

## Appendix B-4: Suggested Format for the Lab-Specific Biosafety Manual

According to federal regulations, all laboratories using biohazardous materials must have a Lab-Specific Biosafety Manual. This manual must be adopted as policy and be accessible to all laboratory personnel. To help the Principal Investigators comply with these regulations, the Biosafety group at ASU has developed documents and templates that should be incorporated into the Lab-Specific Biosafety Manual. Each laboratory should have a binder available that includes the following documents in addition to any supplemental materials the Principal Investigator feels will augment the safe and proper handling of biohazardous materials in their laboratory.

1. [ASU Biosafety Manual](#) – Every laboratory operating at BSL2 or BSL3 must have a copy of this manual available.
2. [NIH Guidelines Research Involving Recombinant DNA](#) – Laboratories that work with recombinant DNA must have a copy of these guidelines available.
3. Institutional Biosafety Committee (IBC) Disclosures – It is recommended that all approved IBC disclosures be made available to laboratory personnel.
4. Biosafety Inspection Reports – EHS performs annual biosafety inspections and provides a report to the Principal Investigator. Please make this report available to laboratory personnel. Principal Investigators may also perform their own self-inspection using the [Biosafety Inspection Checklist](#).
5. [Lab-Specific Biosafety Training Checklist](#) – All personnel in the lab should fill out this document and keep copies available.
6. Biological Inventory – Each laboratory should maintain a list of all biological organisms that may be present, including known hazards for each organism. Biological material safety data sheets (MSDS) or fact sheets provided by the manufacturer should also be provided when available.
7. [HBV Vaccine Declination Forms](#) – Personnel who work with human blood, human blood products, or any unfixed human tissue must be provided Hepatitis B Virus vaccinations, free of charge. If personnel decline the vaccination, the [HBV Vaccine Declination Form](#) must be signed and maintained.
8. Emergency Contacts – A list of all laboratory personnel and contact information should be recorded and made available.
9. Standard Operating Procedures (SOPs) – SOPs relevant to the laboratory must be written and made available to lab personnel. Examples include the [Biosafety Cabinet SOP](#) and [Autoclave Operation SOP](#). Other SOPs may be developed for Emergency Spill Clean-up, Biohazardous Waste Disposal, Accident/Incident Reporting, Transporting Biological Materials, Safe Handling of Sharps, Post-exposure Medical Surveillance, or use of specific equipment in the lab.

Please keep these materials in your laboratory and available to all lab personnel. Annual biosafety inspections will require the laboratory to produce these documents in accordance with federal regulations. If you have any questions about your Lab-Specific Biosafety Manual, please contact [Biosafety@asu.edu](mailto:Biosafety@asu.edu).

## **Appendix C: Special Considerations for Select Agents and Toxins**

### **SELECT AGENT AND TOXIN REGULATORY INFORMATION**

The ASU Biosafety Manual is applicable to all laboratories, research, service and support activities that use or store select agents or toxins that are regulated by DHHS and/or the USDA. The information presented in this manual reflects the requirements and guidelines of federal regulations. Each Principal Investigator will supplement this information with written procedures, instruction and guidance regarding specific practices and procedures unique to the work being done in the laboratory with select agents and toxins.

### **REGULATORY REQUIREMENTS**

#### **Registration Process at ASU**

Arizona State University is prohibited from possessing, using, receiving, or transferring select agents or toxins unless ASU has been granted a certificate of registration by the DHHS Secretary or the USDA Secretary. If a PI from any ASU laboratory would like to apply for a certificate of registration, the PI must contact the [Office for Research Integrity and Assurance](#) (ORIA) and complete the [Proposal Routing and Approval Form](#) in addition to completing the [112C form](#). These are submitted through a process to the IBC, Animal Care (if required), EH&S, and ORIA. The request to conduct research with a select agent is a lengthy one and involves a requirement for a compliant facility as well as completion of a site-specific security plan and an agent specific safety plan. These templates and procedures exist, and the EH&S department is ready to assist a PI to navigate this process.

Once approved by the ORIA/IBC, the ASU Responsible Official (RO) will begin the process to obtain a registration application number from the DHHS or USDA secretary and will submit the application once it has been completed by the RO in conjunction with the PI. The information requested by DHHS or USDA will be used to determine whether or not ASU will be allowed to conduct activities with the select agent or toxin.

Once the application is approved, the certificate of registration will be valid only for the specific select agents and/or toxins, and the specified activities and locations that are consistent with the information provided by ASU upon which the certificate of registration was granted. The RO must promptly notify the DHHS or USDA Secretary, in writing, if a change occurs in any information previously submitted to the DHHS or USDA Secretary. This includes modifications to the list of individuals approved, changes in area of work, protocols, or objectives of studies.

The certificate of registration will cover activities at only one general physical location (a building or a complex of buildings at a single mailing address). Unless terminated sooner, a certificate of registration will be valid for up to three years. To obtain a new certificate of registration, ASU must submit a new application.

#### **Security and Emergency Response Requirements**

Security and emergency response are very important components of the select agents and toxins regulations and must be an integral part of storing and handling these agents and toxins. Requirements regarding the items in the following list can be found in the appendix of this manual titled ASU Laboratory Security Guidance for Laboratories Working with Select Agents:

- Physical security;
- Data and IT system security;
- Security policies for personnel;
- Policies for accessing select agent areas;
- Specimen accountability;
- Receipt of select agents into the laboratory;
- Transfer or shipping of select agents from the laboratory (refer to the previous section);
- Emergency response plans (refer to the section on emergency response procedures);
- Reporting of incidents, injuries, and breaches; and
- Recordkeeping

## **Training**

The PI, in conjunction with the RO, must provide information and training on safety and security for working with biohazardous and select agents and toxins to each individual approved for access to his/her laboratory and each unapproved individual working in, or visiting, areas where select agents and toxins are handled or stored. The PI must ensure that all individuals who work in, or visit, the areas understand the hazards of select agents and toxins present in the area. This training program must be included as part of their Lab-Specific Biosafety Manual and be documented with a signature and date to indicate that each individual was trained. Training records will be kept on file for 3 years.

The PI must provide information and training at the time of an individual's initial assignment to a work area where select agents or toxins are present and prior to assignments involving new exposure situations. The PI must provide refresher training annually and when there are any changes in processes or procedures.

## **Audits and Compliance**

The Responsible Official, or his or her designee, will conduct regular inspections, at least annually, of each laboratory where select agents or toxins are stored or used to ensure compliance with the procedures and protocols of this manual. Inspection reports will document violations and be directed to the PI. The results of these inspections with select agents and toxins will be documented and kept on file with the Responsible Official.

## **Program Evaluation**

Program evaluation is conducted annually based upon the effectiveness of this plan. The Select Agent and Toxin Program measurements must be reviewed, performance tested and updated annually. The plan must also be reviewed and revised as necessary after any incident. Any program evaluation report for laboratories containing select agents and toxins is written by the RO.

**NOTICE PERTINENT TO THE OCTOBER 2011 REVISIONS  
OF THE  
NIH GUIDELINES FOR RESEARCH INVOLVING  
RECOMBINANT DNA MOLECULES  
(NIH GUIDELINES)**

**Summary of Amendments:**

Minor Actions under [Section IV-C-1-b-\(2\)-\(c\)](#): Revising the *Classification of Etiologic Agents* for the purpose of these *NIH Guidelines* (see [Section V-A](#), *Footnotes and References of Sections I-IV*).

Page 13: [Section II-A-3](#) – First paragraph, last sentence: Added a reference to [Appendix B: Classification of Human Etiologic Agents on The Basis of Hazard](#).

Page 39: [Appendix B-II-A](#):  
Added: “--*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4”  
Added: “--*Francisella tularensis* specifically *F. tularensis* subspecies *novocida* [aka *F. novocida*], strain Utah 112; *F. tularensis* subspecies *holartica* LVS; *F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)”  
Added footnote: “\* For research involving high concentrations, BL3 practices should be considered (See Appendix G–II–C–2. Special Practices (BL3)).”

Page 40: Added “--*Yersinia pestis* specifically *pgm*<sup>(-)</sup> strains (lacking the 102 kb pigmentation locus) and *lcr*<sup>(-)</sup> strains (lacking the LCR plasmid)”

Page 41: [Appendix B-II-D](#):  
Added: “--Chikungunya vaccine strain 181/25”  
“--Venezuelan equine encephalomyelitis vaccine strain TC-83” changed to: “--Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526”  
Added: “--Junin virus candid #1 vaccine strain”  
Deleted “(Togaviruses)” from the Flavivirus group (also on page 43)  
Added: “--Japanese encephalitis virus strain SA 14-14-2”  
Corrected: “Papovaviruses” changed to “Papilloma viruses”

Page 42: Changed: “--Vesicular stomatitis virus—laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow” to: “--Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (e.g. Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst)”  
Corrected: “Rubivirus” as a genus name and “Rubella virus” as the type species

Page 43: [Appendix B-III-D](#):  
Added: “--Chikungunya virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)”  
Added: “Coronaviruses” and “--SARS-associated coronavirus (SARS-CoV)”  
Added: “West Nile Virus (WNV)”  
Added references to Appendix B-II-D Risk Group2 (RG2) – Viruses where applicable

Page 44: [Appendix B-V](#)  
Corrected: “Papovaviruses” changed to 1) “Papilloma viruses” (listing Bovine and Shope viruses) and 2) “Polyoma viruses” (listing Polyoma and SV40 viruses)



# NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)

October 2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
National Institutes of Health

\*\*\*\*\*

Visit the OBA Web site at:  
<http://oba.od.nih.gov>

For current information on Guidelines, Protocols, Principal Investigators, Meetings,  
and information about upcoming Gene Therapy Policy Conferences

These *NIH Guidelines* supersede all earlier versions and shall be in effect until further notice.

\*\*\*\*\*

## FEDERAL REGISTER NOTICES

Effective June 24, 1994, Published in Federal Register, July 5, 1994 ([59 FR 34472](#))  
Amendment Effective July 28, 1994, Federal Register, August 5, 1994 ([59 FR 40170](#))  
Amendment Effective April 17, 1995, Federal Register, April 27, 1995 ([60 FR 20726](#))  
Amendment Effective December 14, 1995, Federal Register, January 19, 1996 ([61 FR 1482](#))  
Amendment Effective March 1, 1996, Federal Register, March 12, 1996 ([61 FR 10004](#))  
Amendment Effective January 23, 1997, Federal Register, January 31, 1997 ([62 FR 4782](#))  
Amendment Effective September 30, 1997, Federal Register, October 14, 1997 ([62 FR 53335](#))  
Amendment Effective October 20, 1997, Federal Register, October 29, 1997 ([62 FR 56196](#))  
Amendment Effective October 22, 1997, Federal Register, October 31, 1997 ([62 FR 59032](#))  
Amendment Effective February 4, 1998, Federal Register, February 17, 1998 ([63 FR 8052](#))  
Amendment Effective April 30, 1998, Federal Register, May 11, 1998 ([63 FR 26018](#))  
Amendment Effective April 29, 1999, Federal Register, May 11, 1999 ([64 FR 25361](#))  
Amendment Effective October 2, 2000, Federal Register, October 10, 2000 ([65 FR 60328](#))  
Amendment Effective December 28, 2000 Federal Register, January 5, 2001 ([66 FR 1146](#))  
Amendment Effective December 11, 2001 Federal Register, December 11, 2001 ([66 FR 64051](#))  
Amendment Effective December 19, 2001 Federal Register, November 19, 2001 ([66 FR 57970](#))  
Amendment Effective January 10, 2002 Federal Register, December 11, 2001 ([66 FR 64052](#))  
Amendment Effective January 24, 2002 Federal Register, November 19, 2001 ([66 FR 57970](#))  
Amendment Effective September 22, 2009 Federal Register, September 22, 2009 ([74 FR 48275](#))  
Amendment Effective January 19, 2011 Federal Register, January 19, 2011 ([76 FR 3150](#))  
Amendment Effective May 12, 2011 Federal Register, May 12, 2011 ([76 FR 27653](#))  
Amendment Effective October 11, 2011 Federal Register, October 11, 2011 ([76 FR 62816](#))

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## **SECTION I. SCOPE OF THE NIH GUIDELINES**

### **Section I-A. Purpose**

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-1.** Any recombinant DNA experiment, which according to the *NIH Guidelines* requires approval by NIH, must be submitted to NIH or to another Federal agency that has jurisdiction for review and approval. Once approvals, or other applicable clearances, have been obtained from a Federal agency other than NIH (whether the experiment is referred to that agency by NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval. (See exception in [Section I-A-1-a](#) regarding requirement for human gene transfer protocol registration.)

**Section I-A-1-a.** For experiments 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](#)); Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; and (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

### **Section I-B. Definition of Recombinant DNA Molecules**

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.

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 synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*.

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.

### **Section I-C. General Applicability**

**Section I-C-1.** The *NIH Guidelines* are applicable to:

**Section I-C-1-a.** All recombinant DNA research within the United States (U.S.) or its territories that is within the category of research described in either Section I-C-1-a-(1) or Section I-C-1-a-(2).

**Section I-C-1-a-(1).** Research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines*.

**Section I-C-1-a-(2).** Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

**Section I-C-1-b.** All recombinant DNA research performed abroad that is within the category of research described in either Section I-C-1-b-(1) or Section I-C-1-b-(2).

**Section I-C-1-b-(1).** Research supported by NIH funds.

**Section I-C-1-b-(2).** Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

**Section I-C-1-b-(3).** If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the *NIH Guidelines*.

#### **Section I-D. Compliance with the *NIH Guidelines***

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*.

Information concerning noncompliance with the *NIH Guidelines* may be brought forward by any person. It should be delivered to both NIH/OBA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985, 301-496-9838/301-496-9839 (fax) (for non-USPS mail, use zip code 20817).

In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the *NIH Guidelines*, applicable DHHS and Public Health Service procedures shall govern.

The policies on compliance are as follows:

**Section I-D-1.** All NIH-funded projects involving recombinant DNA techniques must comply with the *NIH Guidelines*. Non-compliance may result in: (i) suspension, limitation, or termination of financial assistance for the noncompliant NIH-funded research project and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

**Section I-D-2.** All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the *NIH Guidelines*. Noncompliance may result in: (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

#### **Section I-E. General Definitions**

The following terms, which are used throughout the *NIH Guidelines*, are defined as follows:

**Section I-E-1.** An "institution" is any public or private entity (including Federal, state, and local government agencies).

**Section I-E-2.** An "Institutional Biosafety Committee" is a committee that: (i) meets the requirements for membership specified in [Section IV-B-2, Institutional Biosafety Committee \(IBC\)](#), and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in [Section IV-B-2, Institutional Biosafety Committee \(IBC\)](#).

**Section I-E-3.** The "Office of Biotechnology Activities (OBA)" is the office within the NIH that is responsible for: (i) reviewing and coordinating all activities relating to the *NIH Guidelines*, and (ii) performing other duties as defined in [Section IV-C-3, Office of Biotechnology Activities \(OBA\)](#).

**Section I-E-4.** The "Recombinant DNA Advisory Committee" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in [Section IV-C-2, Recombinant DNA Advisory Committee \(RAC\)](#).

**Section I-E-5.** The "NIH Director" is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

**Section I-E-6.** "Deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

**Section I-E-7.** "Enrollment" is the process of obtaining informed consent from a potential research participant, or a designated legal guardian of the participant, to undergo a test or procedure associated with the gene transfer experiment.

**Section I-E-8.** A "serious adverse event" is any event occurring at any dose that results in any of the following outcomes: death, a life-threatening event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization also may be considered a serious adverse event when, upon the basis of appropriate medical judgment, they may jeopardize the human gene transfer research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

**Section I-E-9.** 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.

**Section I-E-10.** An "unexpected serious adverse event" is any serious adverse event for which the specificity or severity is not consistent with the risk information available in the current investigator's brochure.

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**SECTION II. SAFETY CONSIDERATIONS**

**Section II-A. Risk Assessment**

**Section II-A-1. Risk Groups**

Risk assessment is ultimately a subjective process. The investigator must make an initial risk assessment based on the Risk Group (RG) of an agent (see [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#)). Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria: (1) Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans. (2) Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available. (3) Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available. (4) Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

## Section II-A-2. Criteria for Risk Groups

Classification of agents in [Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard](#), is based on the potential effect of a biological agent on a healthy human adult and does not account for instances in which an individual may have increased susceptibility to such agents, e.g., preexisting diseases, medications, compromised immunity, pregnancy or breast feeding (which may increase exposure of infants to some agents).

Personnel may need periodic medical surveillance to ascertain fitness to perform certain activities; they may also need to be offered prophylactic vaccines and boosters (see [Section IV-B-1-f, Responsibilities of the Institution, General Information](#)).

## Section II-A-3. Comprehensive Risk Assessment

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 [Appendix B Classification of Human Etiologic Agents on the Basis of Hazard](#) and [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 (BL) 2 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 \(OSHA\)](#) 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 [Sections V-G and V-H, Footnotes and References of Sections I through IV](#).

## Section II-B. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see [Section V-B, Footnotes and References of Sections I-IV](#)). The existing programs rely upon mechanisms that can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in [Appendix G, Physical Containment](#). These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see [Appendix I, Biological Containment](#)).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the *NIH Guidelines*.

Physical containment conditions within laboratories, described in [Appendix G, Physical Containment](#), may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in [Appendix I, Biological Containment](#), may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1-P through BL4-P) are described in [Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants](#). BL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3-P and BL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2-P and BL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4-P describes facilities and practices known to provide containment of certain exotic plant pathogens.



For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in [Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals](#). BL1-N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2-N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3-N and BL4-N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the *NIH Guidelines*, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the *NIH Guidelines* to permit the use of these procedures.

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### **SECTION III. EXPERIMENTS COVERED BY THE NIH GUIDELINES**

This section describes six categories of experiments involving recombinant DNA: (i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see [Section III-A](#)), (ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see [Section III-B](#)), (iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see [Section III-C](#)), (iv) those that require Institutional Biosafety Committee approval before initiation (see [Section III-D](#)), (v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see [Section III-E](#)), and (vi) those that are exempt from the *NIH Guidelines* (see [Section III-F](#)).

**Note:** *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed.* If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.

Any change in containment level, which is different from those specified in the *NIH Guidelines*, may not be initiated without the express approval of NIH/OBA (see [Section IV-C-1-b-\(2\)](#) and its subsections, *Minor Actions*).

**Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH 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.** 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-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation**

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/OBA. The containment conditions for such experiments will be determined by NIH/OBA in consultation with *ad hoc* experts. Such experiments require Institutional Biosafety Committee approval before initiation (see [Section IV-B-2-b-\(1\)](#), *Institutional Biosafety Committee*).

#### **Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms per Kilogram Body Weight**

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD<sub>50</sub> of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section 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-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment**

#### **Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants**

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*).

In its evaluation of human gene transfer proposals, the RAC will consider whether a proposed human gene transfer experiment presents characteristics that warrant public RAC review and discussion (See [Appendix M-I-B-2](#)). The process of public RAC review and discussion is intended to foster the safe and ethical conduct of human gene transfer experiments. Public review and discussion of a human gene transfer experiment (and access to relevant information) also serves to inform the public about the technical aspects of the proposal, meaning and significance of the research, and any significant safety, social, and ethical implications of the research.

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. After a human gene transfer experiment is reviewed by the RAC at a regularly scheduled meeting, NIH OBA will send a letter, unless NIH OBA determines that there are exceptional circumstances, within 10 working days to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate, summarizing the RAC recommendations.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.



In order to maintain public access to information regarding human gene transfer protocols (including protocols that are not publicly reviewed by the RAC), NIH OBA will maintain the documentation described in Appendices M-I through M-V. The information provided in response to [Appendix M](#) should not contain any confidential commercial information or trade secrets, enabling all aspects of RAC review to be open to the public.

**Note:** For specific directives concerning the use of retroviral vectors for gene delivery, consult [Appendix B-V-1, Murine Retroviral Vectors](#).

### **Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation**

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see [Section IV-C-1-b-\(2\)-\(c\), Minor Actions](#)).

#### **Section III-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](#))

**Section III-D-1-a.** Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

**Section III-D-1-b.** Experiments involving the introduction of recombinant DNA into Risk Group 3 agents will usually be conducted at BL3 containment. Experiments with such agents will usually be conducted with whole animals at BL3 or BL3-N containment.

**Section III-D-1-c.** Experiments involving the introduction of recombinant DNA into Risk Group 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

**Section III-D-1-d.** Containment conditions for experiments involving the introduction of recombinant DNA into restricted agents shall be set on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture - [Animal and Plant Health Inspection Service](#) (USDA/APHIS) permit is required for work with plant or animal pathogens (see [Section V-G and V-M, Footnotes and References of Sections I-IV](#)). Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

#### **Section III-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 Systems**

**Section III-D-2-a.** Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see [Section II-A, Risk Assessment](#)) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the *NIH Guidelines* (see [Section III-F, Exempt Experiments](#)). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see [Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms Per Kilogram Body Weight](#)) or shall be conducted under NIH specified conditions as described in [Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#).

**Section III-D-2-b.** Containment conditions for experiments in which DNA from restricted agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/OBA following a case-by-case review (see [Section V-L, Footnotes and References of Sections I-IV](#)). A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G, Footnotes and References of Sections I-IV](#)).

### **Section III-D-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**

**Caution:** Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

**Note:** Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see [Section V-J, Footnotes and References of Sections I-IV](#)) being considered identical (see [Section V-K, Footnotes and References of Sections I-IV](#)), are considered defective and may be used in the absence of helper under the conditions specified in [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](#).

**Section III-D-3-a.** Experiments involving the use of infectious or defective Risk Group 2 viruses (see [Appendix B-II, Risk Group 2 Agents](#)) in the presence of helper virus may be conducted at BL2.

**Section III-D-3-b.** Experiments involving the use of infectious or defective Risk Group 3 viruses (see [Appendix B-III-D, Risk Group 3 \(RG3\) - Viruses and Prions](#)) in the presence of helper virus may be conducted at BL3.

**Section III-D-3-c.** Experiments involving the use of infectious or defective Risk Group 4 viruses (see [Appendix B-IV-D, Risk Group 4 \(RG4\) - Viral Agents](#)) in the presence of helper virus may be conducted at BL4.

**Section III-D-3-d.** Experiments involving the use of infectious or defective restricted poxviruses (see [Sections V-A and V-L, Footnotes and References of Sections I-IV](#)) in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G, Footnotes and References of Sections I-IV](#)).

**Section III-D-3-e.** Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in [Sections III-D-3-a](#) through [III-D-3-d](#) may be conducted at BL1.

### **Section III-D-4. Experiments Involving Whole Animals**

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

**Caution -** Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

**Section III-D-4-a.** Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see [Section V-B, Footnotes and References of Sections I-IV](#)). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under

**Section III-D-4-b, Experiments Involving Whole Animals.** For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see [Sections V-A, V-G, and V-L, Footnotes and References of Sections I-IV](#). It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G, Footnotes and References of Sections I-IV](#)).

**Section III-D-4-b.** For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by [Sections III-D-1, Experiments Using Human or Animal Pathogens \(Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems, or III-D-4-a, Experiments Involving Whole Animals](#), the appropriate containment shall be determined by the Institutional Biosafety Committee.

**Section III-D-4-c.** Exceptions under [Section III-D-4, Experiments Involving Whole Animals](#)

**Section III-D-4-c-(1).** Experiments involving the generation of transgenic rodents that require BL1 containment are described under [Section III-E-3, Experiments Involving Transgenic Rodents](#).

**Section III-D-4-c-(2).** The purchase or transfer of transgenic rodents is exempt from the *NIH Guidelines* under [Section III-F, Exempt Experiments](#) (see [Appendix C-VI, The Purchase or Transfer of Transgenic Rodents](#)).

### **Section III-D-5. Experiments Involving Whole Plants**

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in [Sections III-D-5-a through III-D-5-e](#). If experiments involving whole plants are not described in [Section III-D-5](#) and do not fall under [Sections III-A, III-B, III-D or III-F](#), they are included in [Section III-E](#).

**NOTE** - For recombinant DNA experiments falling under [Sections III-D-5-a through III-D-5-d](#), physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

**Section III-D-5-a.** BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic (see [Section V-M, Footnotes and References of Sections I-IV](#)) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

**Section III-D-5-b.** BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see [Section V-M, Footnotes and References of Sections I-IV](#)) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*.

**Section III-D-5-c.** BL4-P containment is recommended for experiments with a small number of readily transmissible exotic (see [Section V-M, Footnotes and References of Sections I-IV](#)) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

**Section III-D-5-d.** BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD<sub>50</sub> of <100 nanograms per kilogram body weight fall under [Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms Per Kilogram Body Weight](#), and require NIH/OBA and Institutional Biosafety Committee approval before initiation.

**Section III-D-5-e.** BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

### Section III-D-6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, [Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules](#), shall be used. [Appendix K](#) describes containment conditions Good Large Scale Practice through BL3-Large Scale.

### Section III-D-7. Experiments Involving Influenza Viruses

Experiments with influenza viruses generated by recombinant methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the risk group of the virus that was the source of the majority of segments in the recombinant virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3). Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)) unless indicated below.

**Section III-D-7-a. Human H2N2 (1957-1968).** Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BL3 enhanced (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

**Section III-D-7-b. Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).** Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced containment, (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)). Experiments involving influenza viruses containing a minority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at biosafety level 2 and after they have been excluded pursuant to [9 CFR 121.3\(e\)](#). OBA is available to IBCs to provide consultation with the RAC and influenza virus experts when risk assessments are being made to determine the appropriate biocontainment for experiments with influenza viruses containing a minority of gene/segments from HPAI H5N1. Such experiments may be performed at BL3 enhanced containment or containment may be lowered to biosafety level 2, the level of containment for most research with other influenza viruses. ([USDA/APHIS](#) regulations and decisions on lowering containment also apply.) In deciding to lower containment, the IBC should consider whether, in at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates), there is evidence that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. This should be determined by measuring biological indices appropriate for the specific animal model (e.g., severe weight loss, elevated temperature, mortality or neurological symptoms).

**Section III-D-7-c. 1918 H1N1.** Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses](#)).

**Section III-D-7-d. Antiviral Susceptibility and Containment.** The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968). If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc.



Experiments with 1918 H1N1, human H2N2 (1957-1968) or HPAI H5N1 that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational antiviral agents being developed for influenza) would be subject to [Section III-A-1](#) (Major Actions) and require RAC review and NIH Director approval. As per [Section I-A-1](#) of the *NIH Guidelines*, if the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments.

### **Section III-E. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation**

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. For experiments in this category, a registration document (see [Section III-D](#), *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see [Section IV-A](#), *Policy*). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under [Section III-E](#) and may be conducted at BL1 containment.

#### **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.

#### **Section III-E-2. Experiments Involving Whole Plants**

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under [Section III-A](#), [III-B](#), [III-D](#), or [III-F](#). It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

**Section III-E-2-a.** BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in [Section III-E-2-b](#) or other sections of the *NIH Guidelines*. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see [Section V-M](#), *Footnotes and References of Sections I-IV*) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

**Section III-E-2-b.** BL2-P or BL1-P + biological containment is recommended for the following experiments:

**Section III-E-2-b-(1).** Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

**Section III-E-2-b-(2).** Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

**Section III-E-2-b-(3).** Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M, Footnotes and References of Sections I-IV](#)).

**Section III-E-2-b-(4).** Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M, Footnotes and References of Sections I-IV](#)).

**Section III-E-2-b-(5).** Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M, Footnotes and References of Sections I-IV](#)).

**Section III-E-3. Experiments Involving Transgenic Rodents**

This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under [Section III-D-4, Experiments Involving Whole Animals](#).

**Section III-E-3-a.** Experiments involving the breeding of certain BL1 transgenic rodents are exempt under Section III-F, *Exempt Experiments* (See [Appendix C-VII, Generation of BL1 Transgenic Rodents via Breeding](#)).

**Section III-F. Exempt Experiments**

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required:

**Section III-F-1.** Those that are not in organisms or viruses.

**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.

**Section III-F-3.** 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.

**Section III-F-4.** Those that consist entirely of DNA from an 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-5.** 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*.

**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*.

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## SECTION IV. ROLES AND RESPONSIBILITIES

### Section IV-A. Policy

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-B. Responsibilities of the Institution

#### Section IV-B-1. General Information

Each institution conducting or sponsoring recombinant DNA research which is covered by the *NIH Guidelines* is responsible for ensuring that the research is conducted in full conformity with the provisions of the *NIH Guidelines*. In order to fulfill this responsibility, the institution shall:

**Section IV-B-1-a.** Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the *NIH Guidelines*. As part of its general responsibilities for implementing the *NIH Guidelines*, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the *NIH Guidelines*. Such procedures may include: (i) statements formulated by the institution for the general implementation of the *NIH Guidelines*, and (ii) any additional precautionary steps the institution deems appropriate.

**Section IV-B-1-b.** Establish an Institutional Biosafety Committee that meets the requirements set forth in Section IV-B-2-a and carries out the functions detailed in [Section IV-B-2-b](#).

**Section IV-B-1-c.** Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant DNA research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large-scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in [Section IV-B-3](#).

**Section IV-B-1-d.** Appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research Involving Plants*.

**Section IV-B-1-e.** Appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix Q](#), *Physical and Biological Containment for Recombinant DNA Research Involving Animals*.

**Section IV-B-1-f.** Ensure that when the institution participates in or sponsors recombinant DNA research involving human subjects: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary), (ii) all aspects of [Appendix M](#) have been appropriately addressed by the Principal Investigator; and (iii) no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*), Institutional Biosafety Committee approval has been obtained,

Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained. Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

**Section IV-B-1-g.** Assist and ensure compliance with the *NIH Guidelines* by Principal Investigators conducting research at the institution as specified in [Section IV-B-7](#).

**Section IV-B-1-h.** Ensure appropriate training for the Institutional Biosafety Committee Chair and members, Biological Safety Officer and other containment experts (when applicable), Principal Investigators, and laboratory staff regarding laboratory safety and implementation of the *NIH Guidelines*. The Institutional Biosafety Committee Chair is responsible for ensuring that Institutional Biosafety Committee members are appropriately trained. The Principal Investigator is responsible for ensuring that laboratory staff are appropriately trained. The institution is responsible for ensuring that the Principal Investigator has sufficient training; however, this responsibility may be delegated to the Institutional Biosafety Committee.

**Section IV-B-1-i.** Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory. The *Laboratory Safety Monograph* discusses various components of such a program (e.g., records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience). Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples cited in the *Laboratory Safety Monograph* include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the *Laboratory Safety Monograph* are available 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 IV-B-1-j.** Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to NIH/OBA within thirty days, unless the institution determines that a report has already been filed by the Principal Investigator or Institutional Biosafety Committee. Reports shall be sent 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).

## **Section IV-B-2. Institutional Biosafety Committee (IBC)**

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant DNA. The Institutional Biosafety Committee shall meet the following requirements:

### **Section IV-B-2-a. Membership and Procedures**

**Section IV-B-2-a-(1).** The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research*



*Involving Plants*, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing [Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals](#), require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see [Section IV-B-3, Biological Safety Officer](#)). When the institution participates in or sponsors recombinant DNA research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary); (ii) all aspects of [Appendix M](#) have been appropriately addressed by the Principal Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)); and (iv) final IBC approval is granted only after the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)). Institutional Biosafety Committee approval must be obtained from the institution at which recombinant DNA material will be administered to human research participants (rather than the site involved in manufacturing gene transfer products).

**Note:** Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines*, are encouraged to adhere to the standards and procedures set forth in [Sections I](#) through [IV](#) (see [Section IV-D, Voluntary Compliance](#)). The policy and procedures for establishing an Institutional Biosafety Committee under *Voluntary Compliance*, are specified in [Section IV-D-2, Institutional Biosafety Committee Approval](#).

**Section IV-B-2-a-(2).** In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

**Section IV-B-2-a-(3).** The institution shall file an annual report with NIH/OBA which includes: (i) a roster of all Institutional Biosafety Committee members clearly indicating the Chair, contact person, Biological Safety Officer (if applicable), plant expert (if applicable), animal expert (if applicable), human gene therapy expertise or *ad hoc* consultant(if applicable); and (ii) biographical sketches of all Institutional Biosafety Committee members (including community members).

**Section IV-B-2-a-(4).** No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

**Section IV-B-2-a-(5).** The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

**Section IV-B-2-a-(6).** When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

**Section IV-B-2-a-(7).** Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response 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).

#### **Section IV-B-2-b. Functions**

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

**Section IV-B-2-b-(1).** Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* as specified in [Section III, Experiments Covered by the NIH Guidelines](#), and approving those research projects that are found to conform with the *NIH Guidelines*. This review shall include: (i) independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research; (iii) ensuring that all aspects of [Appendix M](#) have been appropriately addressed by the Principal Investigator; (iv) ensuring that no research participant is enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)), Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained; (v) for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principal Investigator's response to the RAC recommendations; (vi) ensuring that final IBC approval is granted only after the RAC review process has been completed (see [Appendix M-I-B, RAC Review Requirements](#)); and (vii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*.

**Section IV-B-2-b-(2).** Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

**Section IV-B-2-b-(3).** Lowering containment levels for certain experiments as specified in [Section III-D-2-a, 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 Systems](#).

**Section IV-B-2-b-(4).** Setting containment levels as specified in [Sections III-D-4-b, Experiments Involving Whole Animals](#), and [III-D-5, Experiments Involving Whole Plants](#).

**Section IV-B-2-b-(5).** Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the *NIH Guidelines*.

**Section IV-B-2-b-(6).** Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

**Note:** The *Laboratory Safety Monograph* describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the *Laboratory Safety Monograph*. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

**Section IV-B-2-b-(7).** Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/OBA shall be sent 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).

**Section IV-B-2-b-(8).** The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the *NIH Guidelines* until NIH (with the advice of the RAC when required) establishes the containment requirement.

**Section IV-B-2-b-(9).** Performing such other functions as may be delegated to the Institutional Biosafety Committee under [Section IV-B-2, Institutional Biosafety Committee](#).

### **Section IV-B-3. Biological Safety Officer (BSO)**

**Section IV-B-3-a.** The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules.

**Section IV-B-3-b.** The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

**Section IV-B-3-c.** The Biological Safety Officer's duties include, but are not be limited to:

**Section IV-B-3-c-(1).** Periodic inspections to ensure that laboratory standards are rigorously followed;

**Section IV-B-3-c-(2).** Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator;

**Section IV-B-3-c-(3).** Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;

**Section IV-B-3-c-(4).** Providing advice on laboratory security;

**Section IV-B-3-c-(5).** Providing technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

**Note:** See the *Laboratory Safety Monograph* for additional information on the duties of the Biological Safety Officer.

#### **Section IV-B-4. Plant, Plant Pathogen, or Plant Pest Containment Expert**

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, the institution shall appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee).

#### **Section IV-B-5. Animal Containment Expert**

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with [Appendix Q](#), *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, the institution shall appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee).

#### **Section IV-B-6. Human Gene Therapy Expertise**

When the institution participates in or sponsors recombinant DNA research involving human subjects, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary) and (ii) all aspects of [Appendix M](#), *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)*, have been appropriately addressed by the Principal Investigator prior to submission to NIH/OBA.

#### **Section IV-B-7. Principal Investigator (PI)**

On behalf of the institution, the Principal Investigator is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research. A Principal Investigator engaged in human gene transfer research may delegate to another party, such as a corporate sponsor, the reporting functions set forth in [Appendix M](#), with written notification to the NIH OBA of the delegation and of the name(s), address, telephone, and fax numbers of the contact. The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

**Section IV-B-7-a. General Responsibilities**

As part of this general responsibility, the Principal Investigator shall:

**Section IV-B-7-a-(1).** Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), and [III-E](#), *Experiments Covered by the NIH Guidelines*) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;

**Section IV-B-7-a-(2).** Determine whether experiments are covered by [Section III-E](#), *Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation*, and ensure that the appropriate procedures are followed;

**Section IV-B-7-a-(3).** Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) within 30 days. Reports to NIH/OBA shall be sent 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);

**Section IV-B-7-a-(4).** Report any new information bearing on the *NIH Guidelines* to the Institutional Biosafety Committee and to NIH/OBA (reports to NIH/OBA shall be sent 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);

**Section IV-B-7-a-(5).** Be adequately trained in good microbiological techniques;

**Section IV-B-7-a-(6).** Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination; and

**Section IV-B-7-a-(7).** Comply with shipping requirements for recombinant DNA molecules (see [Appendix H](#), *Shipment*, for shipping requirements and the *Laboratory Safety Monograph* for technical recommendations).

**Section IV-B-7-b. Information to Be Submitted by the Principal Investigator to NIH OBA**

The Principal Investigator shall:

**Section IV-B-7-b-(1).** Submit information to NIH/OBA for certification of new host-vector systems;

**Section IV-B-7-b-(2).** Petition NIH/OBA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the *NIH Guidelines*;

**Section IV-B-7-b-(3).** Petition NIH/OBA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in [Sections III-A-1](#), *Major Actions Under the NIH Guidelines*, and [III-B](#), *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation*;

**Section IV-B-7-b-(4).** Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and

**Section IV-B-7-b-(5).** Petition NIH/OBA for determination of containment for experiments not covered by the *NIH Guidelines*.

**Section IV-B-7-b-(6).** Ensure that all aspects of [Appendix M](#) have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA, and provide a letter signed by the Principal Investigator(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OBA complies with the requirements set forth in [Appendix M](#). No research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been

completed (see [Appendix M-I-B](#), *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; Institutional Review Board (IRB) approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

#### **Section IV-B-7-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee**

The Principal Investigator shall:

**Section IV-B-7-c-(1).** Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;

**Section IV-B-7-c-(2).** Select appropriate microbiological practices and laboratory techniques to be used for the research;

**Section IV-B-7-c-(3).** Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), or [III-E](#) (*Experiments Covered by the NIH Guidelines*), to the Institutional Biosafety Committee for review and approval or disapproval; and

**Section IV-B-7-c-(4).** Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

#### **Section IV-B-7-d. Responsibilities of the Principal Investigator Prior to Initiating Research**

The Principal Investigator shall:

**Section IV-B-7-d-(1).** Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

**Section IV-B-7-d-(2).** Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents; and

**Section IV-B-7-d-(3).** Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

#### **Section IV-B-7-e. Responsibilities of the Principal Investigator During the Conduct of the Research**

The Principal Investigator shall:

**Section IV-B-7-e-(1).** Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

**Section IV-B-7-e-(2).** Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) (reports to NIH/OBA shall be sent 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);

**Section IV-B-7-e-(3).** Correct work errors and conditions that may result in the release of recombinant DNA materials; and



**Section IV-B-7-e-(4).** Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

**Section IV-B-7-e-(5).** Comply with reporting requirements for human gene transfer experiments conducted in compliance with the *NIH Guidelines* (see [Appendix M-I-C, Reporting Requirements](#)).

## **Section IV-C. Responsibilities of the National Institutes of Health (NIH)**

### **Section IV-C-1. NIH Director**

The NIH Director is responsible for: (i) establishing the *NIH Guidelines*, (ii) overseeing their implementation, and (iii) their final interpretation. The NIH Director has responsibilities under the *NIH Guidelines* that involve OBA and RAC. OBA's responsibilities under the *NIH Guidelines* are administrative. Advice from RAC is primarily scientific, technical, and ethical. In certain circumstances, there is specific opportunity for public comment with published response prior to final action.

#### **Section IV-C-1-a. General Responsibilities**

The NIH Director is responsible for:

**Section IV-C-1-a-(1).** Promulgating requirements as necessary to implement the *NIH Guidelines*;

**Section IV-C-1-a-(2).** Establishing and maintaining RAC to carry out the responsibilities set forth in Section IV-C-2, *Recombinant DNA Advisory Committee* (RAC membership is specified in its [charter](#) and in [Section IV-C-2](#));

**Section IV-C-1-a-(3).** Establishing and maintaining NIH/OBA to carry out the responsibilities defined in [Section IV-C-3, Office of Biotechnology Activities](#);

**Section IV-C-1-a-(4).** Conducting and supporting training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers and other institutional experts (if applicable), Principal Investigators, and laboratory staff.

**Section IV-C-1-a-(5).** Establishing and convening Gene Therapy Policy Conferences as described in [Appendix L, Gene Therapy Policy Conferences](#).

#### **Section IV-C-1-b. Specific Responsibilities**

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the *NIH Guidelines* and presents no significant risk to health or the environment.

##### **Section IV-C-1-b-(1). Major Actions**

To execute *Major Actions*, the NIH Director shall seek the advice of RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and *Proposed Actions* shall be published in the *Federal Register* at least 15 days before the RAC meeting. The NIH Director's decision/recommendation (at his/her discretion) may be published in the *Federal Register* for 15 days of comment before final action is taken. The NIH Director's final decision/recommendation, along with responses to public comments, shall be published in the *Federal Register*. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

**Section IV-C-1-b-(1)-(a).** Changing containment levels for types of experiments that are specified in the *NIH Guidelines* when a *Major Action* is involved;

**Section IV-C-1-b-(1)-(b).** Assigning containment levels for types of experiments that are not explicitly considered in the *NIH Guidelines* when a *Major Action* is involved;

**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 IV-C-1-b-(1)-(d).** Permitting experiments specified by [Section III-A](#), *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation*;

**Section IV-C-1-b-(1)-(e).** Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in [Appendix I-II](#), *Certification of Host-Vector Systems*). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

**Section IV-C-1-b-(1)-(f).** Adopting other changes in the *NIH Guidelines*.

#### **Section IV-C-1-b-(2). Minor Actions**

NIH/OBA shall carry out certain functions as delegated to it by the NIH Director (see [Section IV-C-3](#), *Office of Biotechnology Activities*). *Minor Actions* (as determined by NIH/OBA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to RAC and Institutional Biosafety Committee Chairs:

**Section IV-C-1-b-(2)-(a).** Changing containment levels for experiments that are specified in [Section III](#), *Experiments Covered by the NIH Guidelines* (except when a *Major Action* is involved);

**Section IV-C-1-b-(2)-(b).** Assigning containment levels for experiments not explicitly considered in the *NIH Guidelines*;

**Section IV-C-1-b-(2)-(c).** Revising the *Classification of Etiologic Agents* for the purpose of these *NIH Guidelines* (see [Section V-A](#), *Footnotes and References of Sections I-IV*).

**Section IV-C-1-b-(2)-(d).** Interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels;

**Section IV-C-1-b-(2)-(e).** Setting containment under [Sections III-D-1-d](#), *Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems*, and [III-D-2-b](#), *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 Systems*;

**Section IV-C-1-b-(2)-(f).** Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in [Appendix I-II](#), *Certification of Host-Vector Systems*);

**Section IV-C-1-b-(2)-(g).** Decertifying already certified host-vector systems;

**Section IV-C-1-b-(2)-(h).** Adding new entries to the list of molecules toxic for vertebrates (see [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*); and

**Section IV-C-1-b-(2)-(i).** Determining appropriate containment conditions for experiments according to case precedents developed under [Section IV-C-1-b-\(2\)-\(c\)](#).

## Section IV-C-2. Recombinant DNA Advisory Committee (RAC)

The RAC is responsible for carrying out the functions specified in the *NIH Guidelines*, as well as others specified in its charter or assigned by the Secretary of Health and Human Services or the NIH Director. The RAC membership and procedures, in addition to those set forth in the *NIH Guidelines*, are specified in the charter for the RAC which is filed as provided in the General Services Administration Federal Advisory Committee Management regulations, 41 CFR part 101-6, and is available on the OBA web site, <http://oba.od.nih.gov/oba/rac/RACCharter2009.pdf>. In the event of a conflict between the *NIH Guidelines* and the charter, the charter shall control.

The RAC will consist of not less than 15 voting members, including the Chair, appointed under the procedures of the NIH and the Department of Health and Human Services. The maximum number of voting members will be established in the charter of the RAC. At least a majority of the voting members must be knowledgeable in relevant scientific fields, e.g., molecular genetics, molecular biology, recombinant DNA research, including clinical gene transfer research. At least 4 members of the RAC must be knowledgeable in fields such as public health, laboratory safety, occupational health, protection of human subjects of research, the environment, ethics, law, public attitudes or related fields. Representatives of the Federal agencies listed in the charter shall serve as non-voting members. Nominations for RAC members may be submitted 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).

All meetings of the RAC shall be announced in the *Federal Register*, including tentative agenda items, 15 days before the meeting. Final agendas, if modified, shall be available at least 72 hours before the meeting. No item defined as a *Major Action* under [Section IV-C-1-b-\(1\)](#) may be added to an agenda following *Federal Register* publication.

RAC shall be responsible for:

**Section IV-C-2-a.** Advising the NIH Director on the following actions: (1) Adopting changes in the *NIH Guidelines*. (2) Assigning containment levels, changing containment levels, and approving experiments considered as *Major Actions* under the *NIH Guidelines*, i.e., the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. (3) Promulgating and amending lists 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. (4) Certifying new host-vector systems.

**Section IV-C-2-b.** Identifying novel human gene transfer experiments deserving of public discussion by the full RAC;

**Section IV-C-2-c.** Transmitting to the NIH Director specific comments/ recommendations about: (i) a specific human gene transfer experiment, or (ii) a category of human gene transfer experiments;

**Section IV-C-2-d.** Publicly reviewing human gene transfer clinical trial data and relevant information evaluated and summarized by NIH/OBA in accordance with the annual data reporting requirements;

**Section IV-C-2-e.** Identifying broad scientific, safety, social, and ethical issues relevant to gene therapy research as potential Gene Therapy Policy Conference topics;

**Section IV-C-2-f.** Identifying novel social and ethical issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the preparation of relevant Informed Consent documents; and

**Section IV-C-2-g.** Identifying novel scientific and safety issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the design and submission of human gene transfer clinical trials.



### Section IV-C-3. Office of Biotechnology Activities (OBA)

OBA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. OBA shall carry out such other functions as may be delegated to it by the NIH Director. OBA's responsibilities include (but are not limited to) the following:

**Section IV-C-3-a.** Serving as the focal point for public access to summary information pertaining to human gene transfer experiments;

**Section IV-C-3-b.** Serving as the focal point for data management of human gene transfer experiments;

**Section IV-C-3-c.** Administering the annual data reporting requirements (and subsequent review) for human gene transfer experiments (see [Appendix M-I-C, Reporting Requirements](#));

**Section IV-C-3-d.** Transmitting comments/recommendations arising from public RAC discussion of a novel human gene transfer experiment to the NIH Director. RAC recommendations shall be forwarded to the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate.

**Section IV-C-3-e.** Collaborating with Principal Investigators, Institutional Biosafety Committees, Institutional Review Boards, and other DHHS components (including FDA and the [Office for Human Research Protections](#)), to ensure human gene transfer experiment registration compliance in accordance with [Appendix M-I, Requirements for Protocol Submission, Review, and Reporting-Human Gene Transfer Experiments of the NIH Guidelines](#).

**Section IV-C-3-f.** Administering Gene Therapy Policy Conferences as deemed appropriate by the NIH Director (see [Appendix L, Gene Therapy Policy Conferences](#)).

**Section IV-C-3-g.** Reviewing and approving experiments in conjunction with *ad hoc* experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD<sub>50</sub> of less than or equal to 100 nanograms per kilogram body weight in organisms other than *Escherichia coli* K-12 (see [Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms Per Kilogram Body Weight, Appendix F, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates](#));

**Section IV-C-3-h.** Serving as the executive secretary of RAC;

**Section IV-C-3-i.** Publishing in the *Federal Register*:

**Section IV-C-3-i-(1).** Announcements of RAC meetings and tentative agendas at least 15 days in advance (Note: If the agenda for a RAC meeting is modified, OBA shall make the revised agenda available to anyone upon request in advance of the meeting);

**Section IV-C-3-i-(2).** Announcements of Gene Therapy Policy Conferences and tentative agendas at least 15 days in advance;

**Section IV-C-3-i-(3).** Proposed *Major Actions* (see [Section IV-C-1-b-\(1\), Major Actions](#)) at least 15 days prior to the RAC meeting; and

**Section IV-C-3-j.** Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in [Section IV-B-2, Institutional Biosafety Committee \(IBC\)](#), giving its approval to the Institutional Biosafety Committee membership.

### Section IV-C-4. Other NIH Components

Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

## **Section IV-D. Voluntary Compliance**

### **Section IV-D-1. Basic Policy - Voluntary Compliance**

Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines* are encouraged to follow the standards and procedures set forth in [Sections I](#) through [IV](#). In order to simplify discussion, references hereafter to “institutions” are intended to encompass corporations and individuals who have no organizational affiliation. For purposes of complying with the *NIH Guidelines*, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the *NIH Guidelines*.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures are provided in [Sections IV-D-2](#) through [IV-D-5-b](#), *Voluntary Compliance*, in order to address these concerns.

### **Section IV-D-2. Institutional Biosafety Committee Approval - Voluntary Compliance**

It should be emphasized that employment of an Institutional Biosafety Committee member solely for purposes of membership on the Institutional Biosafety Committee does not itself make the member an institutionally affiliated member. Except for the unaffiliated members, a member of an Institutional Biosafety Committee for an institution not otherwise covered by the *NIH Guidelines* may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. [Section IV-B-2-a\(4\)](#), *Institutional Biosafety Committee*, is modified to that extent for purposes of these institutions.

### **Section IV-D-3. Certification of Host-Vector Systems - Voluntary Compliance**

A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in [Appendix I-II](#), *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under [Section IV-D](#), *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

### **Section IV-D-4. Requests for Exemptions and Approvals - Voluntary Compliance**

Requests for exemptions or other approvals as required by the *NIH Guidelines* should be submitted based on the procedures set forth in [Sections I](#) through [IV](#). In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under [Section IV-D-5-a](#), *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

### **Section IV-D-5. Protection of Proprietary Data - Voluntary Compliance**

#### **Section IV-D-5-a. General**

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, “trade secrets and commercial or financial information that is obtained from a person and that is privileged or confidential.” Under 18 U.S.C. 1905, it is a criminal offense for an officer or employee of the U.S. or any Federal department or agency to publish, divulge, disclose, or make known “in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or) processes...of any person, firm, partnership, corporation, or association.” This provision applies to all employees of the Federal Government, including special Government employees. Members of RAC are “special Government employees.”

In submitting to NIH for purposes of voluntary compliance with the *NIH Guidelines*, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential, commercial, or financial information. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released. If NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised and the actual release will be delayed in accordance with 45 Code of Federal Regulations, Section 5.65(d) and (e).

#### **Section IV-D-5-b. Pre-submission Review**

Any institution not otherwise covered by the *NIH Guidelines*, which is considering submission of data or information voluntarily to NIH, may request pre-submission review of the records involved to determine if NIH will make all or part of the records available upon request under the Freedom of Information Act.

A request for pre-submission review should be submitted to NIH/OBA along with the records involved 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). These records shall be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom on Information Act. NIH/OBA will seek a determination from the responsible official under DHHS regulations (45 CFR Part 5) as to whether the records involved, (or some portion) will be made available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from NIH/OBA files, will be considered records of the institution and not NIH/OBA, and will not be received as part of NIH/OBA files. No copies will be made of such records.

NIH/OBA will inform the institution of the NIH Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of NIH/OBA files. If the institution instructs NIH/OBA to return the records, no copies or summaries of the records will be made or retained by DHHS, NIH, or OBA. The NIH Freedom of Information Officer's determination will represent that official's judgment at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom on Information Act if at the time of the determination the records were in NIH/OBA files and a request was received for such files under the Freedom of Information Act.

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#### **SECTION V. FOOTNOTES AND REFERENCES OF SECTIONS I THROUGH IV**

**Section V-A.** The NIH Director, with advice of the RAC, may revise the classification for the purposes of the *NIH Guidelines* (see [Section IV-C-1-b-\(2\)-\(e\)](#), *Minor Actions*). The revised list of organisms in each risk group is reprinted in [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*.

**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*).

**Section V-C.** U.S. Department of Health and Human Services, Public Health Service, [Centers for Disease Control and Prevention](#) and the [National Institutes of Health](#). *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, 2007. Copies are available from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20401-0001, Phone (202) 512-1800 [<http://www.gpo.gov/>].

**Section V-D.** *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

**Section V-E.** Chin, James ed., *Control of Communicable Diseases Manual*, 17th Edition, 2000. ISBN: 087553-242-X, American Public Health Association, 800 I Street, N.W., Washington, D.C. Phone: (202) 777-2742.

**Section V-F.** *World Health Organization Laboratory Biosafety Manual*, 2nd edition. 1993. WHO Albany, NY. Copies are available from: WHO Publication Centre, USA, (Q Corp) 49 Sheridan Avenue, Albany, New York 12210; Phone: (518) 436-9686 (Order # 1152213).

**Section V-G.** A U.S. Department of Agriculture permit, required for import and interstate transport of plant and animal pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: [Animal and Plant Health Inspection Service \(APHIS\)](#), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

**Section V-H.** American Type Culture Collection Catalogues of plant viruses, animal viruses, cells, bacteria, fungi, etc. are available from American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Phone: (703) 365-2700.

**Section V-I.** U.S. Department of Labor, [Occupational Safety and Health Administration](#), 29 CFR 1910.1030, *Bloodborne Pathogens*. See also, *Exposure to Bloodborne Pathogens*, OSHA 3127, 1996 (Revised).

**Section V-J.** As classified in the *Virus Taxonomy: The Classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, 2000 (0123702003) San Diego, CA.

**Section V-K.** *i.e.*, the total of all genomes within a family shall not exceed two-thirds of the genome.

**Section V-L.** Organisms including alastrim, smallpox (variola) and whitepox may not be studied in the United States except at specified facilities. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, [Centers for Disease Control and Prevention](#), Atlanta, Georgia).

**Section V-M.** In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see [Section V-G](#), *Footnotes and References of Sections I-IV*). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

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**APPENDIX A. EXEMPTIONS UNDER SECTION III-F-5--SUBLISTS OF NATURAL EXCHANGERS**

Certain specified recombinant DNA molecules 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 are exempt from these *NIH Guidelines* (see [Section III-F-5, Exempt Experiments](#)). Institutional Biosafety Committee registration is not required for these exempt experiments. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice from the RAC after appropriate notice and opportunity for public comment (see [Section IV-C-1-b\(1\)\(c\), NIH Director--Specific Responsibilities](#)). For a list of natural exchangers that are exempt from the *NIH Guidelines*, see Appendices A-I through A-VI, *Exemptions Under Section III-F-5 Sublists of Natural Exchangers*. [Section III-F-5, Exempt Experiments](#), describes recombinant DNA molecules that are: (1) composed entirely of DNA segments from one or more of the organisms within a sublist, and (2) to be propagated in any of the organisms within a sublist (see *Classification of Bergey's Manual of Determinative Bacteriology*; 8th edition, R. E. Buchanan and N. E. Gibbons, editors, Williams and Wilkins Company; Baltimore, Maryland 1984). Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (see *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, 2007, U.S. DHHS, Public Health Service, [Centers for Disease Control and Prevention, Atlanta, Georgia](#), and NIH Office of Biosafety, Bethesda, Maryland).

**Appendix A-I. Sublist A**

Genus *Escherichia*  
Genus *Shigella*  
Genus *Salmonella* - including *Arizona*  
Genus *Enterobacter*  
Genus *Citrobacter* - including *Levinea*  
Genus *Klebsiella* - including *oxytoca*  
Genus *Erwinia*  
*Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Pseudomonas mendocina*  
*Serratia marcescens*  
*Yersinia enterocolitica*

**Appendix A-II. Sublist B**

*Bacillus subtilis*  
*Bacillus licheniformis*  
*Bacillus pumilus*  
*Bacillus globigii*  
*Bacillus niger*  
*Bacillus nato*  
*Bacillus amyloliquefaciens*  
*Bacillus atterimus*

**Appendix A-III. Sublist C**

*Streptomyces aureofaciens*  
*Streptomyces rimosus*  
*Streptomyces coelicolor*

**Appendix A-IV. Sublist D**

*Streptomyces griseus*  
*Streptomyces cyaneus*  
*Streptomyces venezuelae*

**Appendix A-V. Sublist E**

One way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

**Appendix A-VI. Sublist F**

- Streptococcus sanguis*
- Streptococcus pneumoniae*
- Streptococcus faecalis*
- Streptococcus pyogenes*
- Streptococcus mutans*

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**APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD**

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, [Biosafety in Microbiological and Biomedical Laboratories](#) (see [Sections V-C, V-D, V-E, and V-F](#), [Footnotes and References of Sections I through IV](#)). Further guidance on agents not listed in Appendix B may be obtained through: [Centers for Disease Control and Prevention](#), Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the *NIH Guidelines*.

**Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)**

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)

**Appendix B-I. Risk Group 1 (RG1) Agents**

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see [Appendix C-IV-A, Bacillus subtilis or Bacillus licheniformis Host-Vector Systems, Exceptions](#)); adeno- associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (see [Appendix C-II-A, Escherichia coli K-12 Host Vector Systems, Exceptions](#)) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen); and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.



Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

## Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

### Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

- Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
- Actinobacillus*
- Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- Aeromonas hydrophila*
- Amycolata autotrophica*
- Archaeobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- Arizona hinshawii* - all serotypes
- Bacillus anthracis*
- Bartonella henselae*, *B. quintana*, *B. vinsonii*
- Bordetella* including *B. pertussis*
- Borrelia recurrentis*, *B. burgdorferi*
- Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3)
- Campylobacter coli*, *C. fetus*, *C. jejuni*
- Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- Clostridium botulinum*, *C. chauvoei*, *C. haemolyticum*, *C. histolyticum*, *C. novyi*, *C. septicum*, *C. tetani*
- Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
- Corynebacterium diphtheriae*, *C. pseudotuberculosis*, *C. renale*
- Dermatophilus congolensis*
- Edwardsiella tarda*
- Erysipelothrix rhusiopathiae*
- Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- \**Francisella tularensis* specifically \**F. tularensis* subspecies *novocida* [aka *F. novocida*], strain Utah 112; \**F. tularensis* subspecies *holartica* LVS; \**F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)
- Haemophilus ducreyi*, *H. influenzae*
- Helicobacter pylori*
- Klebsiella* - all species except *K. oxytoca* (RG1)
- Legionella* including *L. pneumophila*
- Leptospira interrogans* - all serotypes
- Listeria*
- Moraxella*
- Mycobacterium* (except those listed in [Appendix B-III-A](#) (RG3)) including *M. avium* complex, *M. asiaticum*, *M. bovis* BCG vaccine strain, *M. chelonae*, *M. fortuitum*, *M. kansasii*, *M. leprae*, *M. malmoense*, *M. marinum*, *M. paratuberculosis*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, *M. xenopi*
- Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- Neisseria gonorrhoeae*, *N. meningitidis*
- Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- Rhodococcus equi*
- Salmonella* including *S. arizonae*, *S. choleraesuis*, *S. enteritidis*, *S. gallinarum-pullorum*, *S. meleagridis*, *S. paratyphi*, A, B, C, *S. typhi*, *S. typhimurium*
- Shigella* including *S. boydii*, *S. dysenteriae*, type 1, *S. flexneri*, *S. sonnei*
- Sphaerophorus necrophorus*
- Staphylococcus aureus*
- Streptobacillus moniliformis*

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\* For research involving high concentrations, BL3 practices should be considered (See [Appendix G-II-C-2. Special Practices](#) (BL3)).

- Streptococcus* including *S. pneumoniae*, *S. pyogenes*
- Treponema pallidum*, *T. carateum*
- Vibrio cholerae*, *V. parahemolyticus*, *V. vulnificus*
- Yersinia enterocolitica*
- Yersinia pestis* specifically *pgm*<sup>(-)</sup> strains (lacking the 102 kb pigmentation locus) and *lcr*<sup>(-)</sup> strains (lacking the LCR plasmid).

#### Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents

- Blastomyces dermatitidis*
- Cladosporium bantianum*, *C. (Xylohypha) trichoides*
- Cryptococcus neoformans*
- Dactylaria galopava* (*Ochroconis gallopavum*)
- Epidermophyton*
- Exophiala (Wangiella) dermatitidis*
- Fonsecaea pedrosoi*
- Microsporum*
- Paracoccidioides braziliensis*
- Penicillium marneffeii*
- Sporothrix schenckii*
- Trichophyton*

#### Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents

- Ancylostoma* human hookworms including *A. duodenale*, *A. ceylanicum*
- Ascaris* including *Ascaris lumbricoides suum*
- Babesia* including *B. divergens*, *B. microti*
- Brugia* filaria worms including *B. malayi*, *B. timori*
- Coccidia*
- Cryptosporidium* including *C. parvum*
- Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
- Echinococcus* including *E. granulosus*, *E. multilocularis*, *E. vogeli*
- Entamoeba histolytica*
- Enterobius*
- Fasciola* including *F. gigantica*, *F. hepatica*
- Giardia* including *G. lamblia*
- Heterophyes*
- Hymenolepis* including *H. diminuta*, *H. nana*
- Isospora*
- Leishmania* including *L. braziliensis*, *L. donovani*, *L. ethiopia*, *L. major*, *L. mexicana*, *L. peruviana*, *L. tropica*
- Loa loa* filaria worms
- Microsporidium*
- Naegleria fowleri*
- Necator* human hookworms including *N. americanus*
- Onchocerca* filaria worms including, *O. volvulus*
- Plasmodium* including simian species, *P. cynomologi*, *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*
- Sarcocystis* including *S. sui hominis*
- Schistosoma* including *S. haematobium*, *S. intercalatum*, *S. japonicum*, *S. mansoni*, *S. mekongi*
- Strongyloides* including *S. stercoralis*
- Taenia solium*
- Toxocara* including *T. canis*
- Toxoplasma* including *T. gondii*
- Trichinella spiralis*
- Trypanosoma* including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. cruzi*
- Wuchereria bancrofti* filaria worms

#### Appendix B-II-D. Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types



Alphaviruses (Togaviruses) - Group A Arboviruses

- Chikungunya vaccine strain 181/25
- Eastern equine encephalomyelitis virus
- Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
- Western equine encephalomyelitis virus

Arenaviruses

- Junin virus candid #1 vaccine strain
- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source (see [Section V-C](#), *Footnotes and References of Sections I through IV*)

Bunyaviruses

- Bunyamwera virus
- Rift Valley fever virus vaccine strain MP-12
- Other viruses as listed in the reference source (see [Section V-C](#), *Footnotes and References of Sections I through IV*)

Caliciviruses

Coronaviruses

Flaviviruses - Group B Arboviruses

- Dengue virus serotypes 1, 2, 3, and 4
- Japanese encephalitis virus strain SA 14-14-2
- Yellow fever virus vaccine strain 17D
- Other viruses as listed in the reference source (see [Section V-C](#), *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see [Appendix B-IV-D](#), *Risk Group 4 (RG4) - Viral Agents*)

- Cytomegalovirus
- Epstein Barr virus
- Herpes simplex* types 1 and 2
- Herpes zoster*
- Human herpesvirus types 6 and 7

Orthomyxoviruses

- Influenza viruses types A, B, and C (except those listed in [Appendix B-III-D](#), *Risk Group 3 (RG3) - Viruses and Prions*)
- Tick-borne orthomyxoviruses

Papilloma viruses

- All human papilloma viruses

Paramyxoviruses

- Newcastle disease virus
- Measles virus
- Mumps virus
- Parainfluenza viruses types 1, 2, 3, and 4
- Respiratory syncytial virus

Parvoviruses

- Human parvovirus (B19)

**Picornaviruses**

- Coxsackie viruses types A and B
- Echoviruses - all types
- Polioviruses - all types, wild and attenuated
- Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see [Appendix B-III-D, Risk Group 3 \(RG3\) - Viruses and Prions](#)) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see [Section V-L, Footnotes and References of Sections I through IV](#))

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

**Rhabdoviruses**

- Rabies virus - all strains
- Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (e.g. Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst)

**Rubivirus (Togaviruses)**

- Rubella virus

**Appendix B-III. Risk Group 3 (RG3) Agents**

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

**Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia**

- Bartonella*
- Brucella* including *B. abortus*, *B. canis*, *B. suis*
- Burkholderia (Pseudomonas) mallei*, *B. pseudomallei*
- Coxiella burnetii* (except the Phase II, Nine Mile strain listed in [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#))
- Francisella tularensis* (except those strains listed in [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#))
- Mycobacterium bovis* (except BCG strain, see [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#)), *M. tuberculosis*
- Pasteurella multocida* type B - "buffalo" and other virulent strains
- Rickettsia akari*, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R. siberica*, *R. tsutsugamushi*, *R. typhi* (*R. mooseri*)
- Yersinia pestis* (except those strains listed in [Appendix B-II-A, Risk Group 2 \(RG2\) - Bacterial Agents Including Chlamydia](#))

**Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents**

- Coccidioides immitis* (sporulating cultures; contaminated soil)
- Histoplasma capsulatum*, *H. capsulatum* var. *duboisii*

**Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents**

None

**Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions**

Alphaviruses (Togaviruses) - Group A Arboviruses

- Chikungunya virus (except the vaccine strain 181/25 listed in [Appendix B-II-D Risk Group2 \(RG2\) – Viruses](#))
- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see [Appendix B-II-D \(RG2\) – Viruses](#))

--Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Arenaviruses

- Flexal
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Coronaviruses

- SARS-associated coronavirus (SARS-CoV)

Flaviviruses - Group B Arboviruses

- Japanese encephalitis virus (except those strains listed in [Appendix B-II-D Risk Group2 \(RG2\) - Viruses](#))
- West Nile virus (WNV)
- Yellow fever virus
- Other viruses as listed in the reference source (see [Section V-C, Footnotes and References of Sections I through IV](#))

Orthomyxoviruses

- Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses

- Monkeypox virus

Prions

- Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see [Section V-C, Footnotes and References of Sections I through IV](#), for containment instruction)

Retroviruses

- Human immunodeficiency virus (HIV) types 1 and 2
- Human T cell lymphotropic virus (HTLV) types 1 and 2
- Simian immunodeficiency virus (SIV)

Rhabdoviruses

- Vesicular stomatitis virus (except those strains listed in [Appendix B-II-D Risk Group2 \(RG2\) - Viruses](#))

**Appendix B-IV. Risk Group 4 (RG4) Agents**

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

**Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents**

None

**Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents**

None

**Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents**

None

**Appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents**

Arenaviruses

- Guanarito virus
- Lassa virus

- Junin virus (except the candid #1 vaccine strain listed in [Appendix B-II-D Risk Group2 \(RG2\) – Viruses](#))
- Machupo virus
- Sabia

Bunyaviruses (Nairovirus)

- Crimean-Congo hemorrhagic fever virus

Filoviruses

- Ebola virus
- Marburg virus

Flaviruses - Group B Arboviruses

- Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)

- Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses

- Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

#### **Appendix B-V. Animal Viral Etiologic Agents in Common Use**

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses

- Herpesvirus ateles
- Herpesvirus saimiri
- Marek's disease virus
- Murine cytomegalovirus

Papilloma viruses

- Bovine papilloma virus
- Shope papilloma virus

Polyoma viruses

- Polyoma virus
- Simian virus 40 (SV40)

Retroviruses

- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus

--Mouse mammary tumor virus  
 --Murine leukemia virus

--Murine sarcoma virus  
 --Rat leukemia virus

### 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.

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## APPENDIX C. EXEMPTIONS UNDER SECTION III-F-6

Section III-F-6 states that exempt from these *NIH Guidelines* are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*), 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 Sections III-F-6*, for other classes of experiments which are exempt from the *NIH Guidelines*." The following classes of experiments are exempt under Section III-F-6:

### Appendix C-I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see Appendix C-VIII-E, *Footnotes and References of Appendix C*), that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed in Appendix C-I-A.

#### Appendix C-I-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents, (iii) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*), and (iv) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

### Appendix C-II. *Escherichia coli* K-12 Host-Vector Systems

Experiments which use *Escherichia coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the *NIH Guidelines* provided that: (i) the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-VIII-B, *Footnotes and References of Appendix C*) shall be used as vectors. However, experiments involving the insertion into *Escherichia coli* K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-VIII-C, *Footnotes and References of Appendix C*) with *Escherichia coli* may be performed with any *Escherichia coli* K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *Escherichia coli* K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

#### Appendix C-II-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-B

which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B](#), *Classification of Human*

*Etiologic Agents on the Basis of Hazard*, and [Sections V-G and V-L](#), *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

### **Appendix C-III. *Saccharomyces* Host-Vector Systems**

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the *NIH Guidelines*. For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

#### **Appendix C-III-A. Exceptions**

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#) which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*, and [Sections V-G and V-L](#), *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

### **Appendix C-IV. *Kluyveromyces* Host-Vector Systems**

Experiments involving *Kluyveromyces lactis* host-vector systems, with the exception of experiments listed in Appendix C-IV-A, are exempt from the *NIH Guidelines* provided laboratory-adapted strains are used (i.e. strains that have been adapted to growth under optimal or defined laboratory conditions). For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee may specify higher containment if deemed necessary.

#### **Appendix C-IV-A Exceptions**

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#), which require NIH/OBA and Institutional Biosafety Committee approval before initiation; (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*, and [Sections V-G and V-L](#), *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval; (iii) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

### **Appendix C-V. *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems**

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than  $10^{-7}$  may be used for cloning DNA with the exception of those experiments listed in Appendix C-V-A, *Exceptions*. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment

conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

#### **Appendix C-V-A. Exceptions**

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#) which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*, and [Sections V-G and V-L](#), *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

#### **Appendix C-VI. Extrachromosomal Elements of Gram Positive Organisms**

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in [Appendix C](#)), propagated and maintained in organisms listed below are exempt from these *NIH Guidelines*.

*Bacillus amyloliquefaciens*  
*Bacillus amylosacchariticus*  
*Bacillus anthracis*  
*Bacillus atterimus*  
*Bacillus brevis*  
*Bacillus cereus*  
*Bacillus globigii*  
*Bacillus licheniformis*  
*Bacillus megaterium*  
*Bacillus natto*  
*Bacillus niger*  
*Bacillus pumilus*  
*Bacillus sphaericus*  
*Bacillus stearothermophilis*  
*Bacillus subtilis*  
*Bacillus thuringiensis*  
*Clostridium acetobutylicum*  
*Lactobacillus casei*  
*Listeria grayi*  
*Listeria monocytogenes*  
*Listeria murrayi*  
*Pediococcus acidilactici*  
*Pediococcus damnosus*  
*Pediococcus pentosaceus*  
*Staphylococcus aureus*  
*Staphylococcus carnosus*  
*Staphylococcus epidermidis*  
*Streptococcus agalactiae*  
*Streptococcus anginosus*  
*Streptococcus avium*  
*Streptococcus cremoris*  
*Streptococcus dorans*  
*Streptococcus equisimilis*  
*Streptococcus faecalis*  
*Streptococcus ferus*  
*Streptococcus lactis*  
*Streptococcus ferns*  
*Streptococcus mitior*  
*Streptococcus mutans*



*Streptococcus pneumoniae*  
*Streptococcus pyogenes*

*Streptococcus salivarius*  
*Streptococcus sanguis*  
*Streptococcus sobrinus*  
*Streptococcus thermophilus*

**Appendix C-VI-A. Exceptions**

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in [Section III-B](#) which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (ii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*, and [Sections V-G and V-L](#), *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in [Section III-D-2](#) with prior Institutional Biosafety Committee review and approval, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

**Appendix C-VII. The Purchase or Transfer of Transgenic Rodents**

The purchase or transfer of transgenic rodents for experiments that require BL1 containment (See [Appendix G-III-M](#), *Footnotes and References of Appendix G*) are exempt from the *NIH Guidelines*.

**Appendix C-VIII. Generation of BL1 Transgenic Rodents via Breeding**

The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the *NIH Guidelines* if:

- (1) Both parental rodents can be housed under BL1 containment; and
- (2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
- (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

**Appendix C-IX. Footnotes and References of Appendix C**

**Appendix C-IX-A.** The NIH Director, with advice of the RAC, may revise the classification for the purposes of these *NIH Guidelines* (see [Section IV-C-1-b-\(2\)-\(b\)](#), *Minor Actions*). The revised list of organisms in each Risk Group is located in [Appendix B](#).

**Appendix C-IX-B.** A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

**Appendix C-IX-C.** Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under [Section III-F-5](#), *Exempt Experiments*.

**Appendix C-IX-D.** As classified in the *Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses*, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

**Appendix C-IX-E.** i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

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## APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE NIH GUIDELINES

As noted in the subsections of [Section IV-C-1-b-\(1\)](#), the Director, NIH, may take certain actions with regard to the *NIH Guidelines* after the issues have been considered by the RAC. Some of the actions taken to date include the following:

**Appendix D-1.** Permission is granted to clone foot and mouth disease virus in the EK1 host-vector system consisting of *E. coli* K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

**Appendix D-2.** Certain specified clones derived from segments of the foot and mouth disease virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech, Inc., has been approved under BL1 + EK1 conditions.

**Appendix D-3.** The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K-12 employing the non-conjugative *Hemophilus* plasmid, pRSF0885, under BL1 conditions.

**Appendix D-4.** Permission is granted to clone certain subgenomic segments of foot and mouth disease virus in HV1 *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems under BL1 conditions at Genentech, Inc., South San Francisco, California.

**Appendix D-5.** Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

**Appendix D-6.** Permission is granted to clone in *E. coli* K-12 under BL1 physical containment conditions subgenomic segments of rift valley fever virus subject to conditions which have been set forth by the RAC.

**Appendix D-7.** Attenuated laboratory strains of *Salmonella typhimurium* may be used under BL1 physical containment conditions to screen for the *Saccharomyces cerevisiae* pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

**Appendix D-8.** Permission is granted to transfer certain clones of subgenomic segments of foot and mouth disease virus from Plum Island Animal Disease Center to the laboratories of Molecular Genetics, Inc., Minnetonka, Minnesota, and to work with these clones under BL1 containment conditions. Approval is contingent upon review of data on infectivity testing of the clones by a working group of the RAC.

**Appendix D-9.** Permission is granted to Dr. John Sanford of Cornell University to field test tomato and tobacco plants transformed with bacterial (*E. coli* K-12) and yeast DNA using pollen as a vector.

**Appendix D-10.** Permission is granted to Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to release under specified conditions *Pseudomonas syringae*, pathovars (pv.) *syringae*, and *Erwinia herbicola* carrying *in vitro* generated deletions of all or part of the genes involved in ice nucleation.

**Appendix D-11.** Agracetus of Middleton, Wisconsin, may field test under specified conditions disease resistant tobacco plants prepared by recombinant DNA techniques.

**Appendix D-12.** Eli Lilly and Company of Indianapolis, Indiana, may conduct large-scale experiments and production involving *Cephalosporium acremonium* strain LU4-79-6 under less than Biosafety Level 1 - Large Scale (BL1-LS) conditions.

**Appendix D-13.** Drs. W. French Anderson, R. Michael Blaese, and Steven Rosenberg of the NIH, Bethesda, Maryland, can conduct experiments in which a bacterial gene coding for neomycin phosphotransferase will be inserted into a portion of the tumor infiltrating lymphocytes (TIL) of cancer patients using a retroviral vector, N2. The marked TIL then will be combined with unmarked TIL, and reinfused into the patients. This experiment is an addition to an ongoing adoptive immunotherapy protocol in which TIL are isolated from a patient's tumor, grown in culture in the presence of interleukin-2, and reinfused into the patient. The marker gene will be used to detect TIL at various time intervals following reinfusion.

Approval is based on the following four stipulations: (i) there will be no limitation of the number of patients in the continuing trial; (ii) the patients selected will have a life expectancy of about 90 days; (iii) the patients give fully informed consent to participate in the trial; and (iv) the investigators will provide additional data before inserting a gene for therapeutic purposes. (Protocol #8810-001)

**Appendix D-14.** U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) may conduct certain experiments involving products of a yellow fever virus originating from the 17-D yellow fever clone at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

In addition, USAMRIID may conduct certain experiments involving vaccine studies of Venezuelan equine encephalitis virus at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

**Appendix D-15.** Drs. R. Michael Blaese and W. French Anderson of the NIH, Bethesda, Maryland, can conduct experiments in which a gene coding for adenosine deaminase (ADA) will be inserted into T lymphocytes of patients with severe combined immunodeficiency disease, using a retroviral vector, LASN. Following insertion of the gene, these T lymphocytes will be reinfused into the patients. The patients will then be followed for evidence of clinical improvement in the disease state, and measurement of multiple parameters of immune function by laboratory testing.

Approval is based on the following two stipulations: (i) that intraperitoneal administration of transduced T lymphocytes not be used before clearance by the Chair of the Recombinant DNA Advisory Committee; and (ii) that the number of research patients be limited to 10 at this time.

In addition to the conditions outlined in the initial approval, patients may be given a supplement of a CD-34+-enriched peripheral blood lymphocytes (PBL) which have been placed in culture conditions that favor progenitor cell growth. This enriched population of cells will be transduced with the retroviral vector, G1NaSvAd. G1NaSvAd is similar to LASN, yet distinguishable by PCR. LASN has been used to transduce peripheral blood T lymphocytes with the ADA gene. Lymphocytes and myeloid cells will be isolated from patients over time and assayed for the presence of the LASN or G1NaSvAd vectors. The primary objectives of this protocol are to transduce CD 34+ peripheral blood cells with the adenosine deaminase gene, administer these cells to patients, and determine if such cells can differentiate into lymphoid and myeloid cells *in vivo*. There is a potential for benefit to the patients in that these hematopoietic progenitor cells may survive longer, and divide to yield a broader range of gene-corrected cells. (Protocol #9007-002)

**Appendix D-16.** Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma who have failed all effective therapy. These patients will be treated with escalating doses of autologous tumor infiltrating lymphocytes (TIL) transduced with a gene coding for tumor necrosis factor (TNF). Escalating numbers of transduced TIL will be administered at three weekly intervals along with the administration of interleukin-2 (IL-2). The objective is to evaluate the toxicity and possible therapeutic efficacy of the administration of tumor infiltrating lymphocytes (TIL) transduced with the gene coding for TNF. (Protocol #9007-003)

**Appendix D-17.** Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on patients with acute myelogenous leukemia (AML). Using the LNL6 retroviral vector, the autologous bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of this gene marking experiment is to determine whether the source of relapse after autologous bone marrow transplantation for acute myelogenous leukemia is residual malignant cells in the harvested marrow or reoccurrence of tumor in the patient. Determining the source of relapse should indicate whether or not purging of the bone marrow is a necessary procedure. (Protocol #9102-004)

**Appendix D-18.** Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on pediatric patients with Stage D (disseminated) neuroblastoma who are being treated with high-dose carboplatin and etoposide in either phase I/II or phase II trials. All the patients in these studies will be subjected to bone marrow transplantation since it will allow them to be exposed to chemoradiation that would be lethal were it not for the availability of stored autologous marrow for rescue. The bone marrow cells of these patients will be transduced with the gene coding for neomycin resistance using the LNL6 vector. The purpose of this gene marking study is to determine whether the source of relapse after autologous bone marrow transplantation is residual malignant cells in the harvested marrow or residual disease in the patient. Secondly,

it is hoped to determine the contribution of marrow autographs to autologous reconstitution. (Protocol #9105-005/9105-006)

**Appendix D-19.** Dr. Albert B. Deisseroth of the MD Anderson Cancer Center of Houston, Texas, can conduct experiments on patients with chronic myelogenous leukemia who have been reinduced into a second chronic phase or blast cells. The patients in these studies will receive autologous bone marrow transplantation. Using the LNL6 vector, the bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of these marking studies is to determine if the origin of relapse arises from residual leukemic cells in the patients or from viable leukemic cells remaining in the bone marrow used for autologous transplantation. (Protocol #9105-007)

**Appendix D-20.** Drs. Fred D. Ledley and Savio L. C. Woo of Baylor College of Medicine of Houston, Texas, can conduct experiments on pediatric patients with acute hepatic failure who are identified as candidates for hepatocellular transplantation. Using the LNL6 vector, the hepatocytes will be transduced with the gene coding for neomycin resistance. The purpose of using a genetic marker is to demonstrate the pattern of engraftment of transplanted hepatocytes and to help determine the success or failure of engraftment. (Protocol #9105-008)

**Appendix D-21.** Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma, renal cell cancer, and colon carcinoma who have failed all effective therapy. In an attempt to increase these patients' immune responses to the tumor, the tumor necrosis factor gene or the interleukin-2 gene will be introduced into a tumor cell line established from the patient. These gene-modified autologous tumor cells will then be injected into the thigh of the patient. To further utilize the immune system of the patient to fight the tumor, stimulated lymphocytes will be cultured from either the draining regional lymph nodes or the injected tumor itself. The patients will be evaluated for antitumor effects engendered by the injection of the gene modified tumor cells themselves as well as after the infusion of the cultured lymphocytes. (Protocol #9110-010/9110-011)

**Appendix D-22.** Dr. James M. Wilson of the University of Michigan Medical Center of Ann Arbor, Michigan, can conduct experiments on three patients with the homozygous form of familial hypercholesterolemia. Both children and adults will be eligible for this therapy. In an attempt to correct the basic genetic defect in this disease, the gene coding for the low-density lipoprotein (LDL) receptor will be introduced into liver cells taken from the patient. The gene-corrected hepatocytes will then be infused into the portal circulation of the patient through an indwelling catheter. The patients will be evaluated for engraftment of these treated hepatocytes through a series of metabolic studies; three months after gene therapy, a liver biopsy will be taken and analyzed for the presence of recombinant derived RNA and DNA to document the presence of the gene coding for the normal LDL receptor. (Protocol #9110-012)

**Appendix D-23.** Dr. Michael T. Lotze of the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, can conduct experiments on 20 patients with metastatic melanoma who have failed conventional therapy. A gene transfer experiment will be performed, transducing the patients' tumor infiltrating lymphocytes (TILs) with the gene for neomycin resistance. Through the use of this gene marking technique, it is proposed to determine how long TIL cells can be detected *in vivo* in the peripheral blood of the patients, and how the administration of interleukin-2 and interleukin-4 affects localization and survival of TIL cells in tumor sites. (Protocol #9105-009)

**Appendix D-24.** Dr. Gary J. Nabel of the University of Michigan Medical School, Ann Arbor, Michigan, can conduct gene therapy experiments on twelve patients with melanoma or adenocarcinoma. Patient population will be limited to adults over the age of 18 and female patients must be postmenopausal or have undergone tubal ligation or orchiectomy. The patient's immune response will be stimulated by the introduction of a gene encoding for a Class I MHC protein, HLA-B7, in order to enhance tumor regression. DNA/liposome-mediated transfection techniques will be used to directly transfer this foreign gene into tumor cells. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. These experiments will be analyzed for their efficacy in treating cancer. (Protocol #9202-013)

**Appendix D-25.** Kenneth Cornetta of Indiana University, Indianapolis, Indiana, can conduct gene transfer experiments on up to 10 patients with acute myelogenous leukemia (AML) and up to 10 patients with acute lymphocytic leukemia (ALL). The patient population will be limited to persons between 18 and 65 years of age. Using the LNL-6 vector, autologous bone marrow cells will be marked with the neomycin resistance gene. Gene marked and untreated bone marrow cells will be reinfused at the time of bone marrow transplantation. Patients

will then be monitored for evidence of the neomycin resistance gene in peripheral blood and bone marrow cells in order to determine whether relapse of their disease is a result of residual malignant cells remaining in the harvested marrow or inadequate ablation of the tumor cells by chemotherapeutic agents. Determining the source of relapse may indicate whether or not purging of the bone marrow is a necessary procedure for these leukemia patients. Further studies will be performed in order to determine the percentage of leukemic cells that contain the LNL-6 vector and the clonality of the marked cells. (Protocol #9202-014)

**Appendix D-26.** Dr. James S. Economou of the University of California, Los Angeles, can conduct gene transfer experiments on 20 patients with metastatic melanoma and 20 patients with renal cell carcinoma. These patients will be treated with various combinations of tumor-infiltrating lymphocytes and peripheral blood leukocytes, including CD8 and CD4 subsets of both types of cells. These effector cell populations will be given in combination with interleukin-2 (IL-2) in the melanoma patients and IL-2 plus alpha interferon in the renal cell carcinoma patients. The effector cells will be transduced with the neomycin resistance gene using either the LNL6 or G1N retroviral vectors. This "genetic marking" of the tumor-infiltrating lymphocytes and peripheral blood lymphocytes is designed to answer questions about the trafficking of these cells, their localization to tumors, and their *in vivo* life span. (Protocol #9202-015)

**Appendix D-27.** Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 15 human immunodeficiency virus (HIV) seropositive patients (18-45 years old) undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma and 15 HIV-seropositive patients (18-50 years old) who do not have acquired immunodeficiency syndrome (AIDS)-related lymphoma and who are not undergoing bone marrow transplantation to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV-TK) gene. This vector will deliver both a marker gene and an ablatable gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of *in vivo* survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells. (Protocol #9202-017)

**Appendix D-28.** Dr. Malcolm Brenner of St. Jude Children's Research Hospital, Memphis, Tennessee, can conduct gene therapy experiments on twelve patients with relapsed/refractory neuroblastoma who have relapsed after receiving autologous bone marrow transplant. In an attempt to stimulate the patient's immune response, the gene coding for Interleukin-2 (IL-2) will be used to transduce tumor cells, and these gene-modified cells will be injected subcutaneously in a Phase 1 dose escalation trial. Patients will be evaluated for an anti-tumor response. (Protocol #9206-018)

**Appendix D-29.** Drs. Edward Oldfield, Kenneth Culver, Zvi Ram, and R. Michael Blaese of the National Institutes of Health, Bethesda, Maryland, can conduct gene therapy experiments on ten patients with primary malignant brain tumors and ten patients with lung cancer, breast cancer, malignant melanoma, or renal cell carcinoma who have brain metastases. The patient population will be limited to adults over the age of 18. Patients will be divided into two groups based on the surgical accessibility of their lesions. Both surgically accessible and surgically inaccessible lesions will receive intra-tumoral injections of the retroviral Herpes simplex thymidine kinase (HS-tk) vector-producer cell line, G1TkSvNa, using a guided stereotaxic approach. Surgically accessible lesions will be excised seven days after stereotaxic injection, and the tumor bed will be infiltrated with the HS-tk producer cells. The removed tumor will be evaluated for the efficiency of transduction. Ganciclovir (GCV) will be administered beginning on the fifth postoperative day. In the case of surgically inaccessible lesions, the patients will receive intravenous therapy with GCV seven days after receiving the intra-tumoral injections of the retroviral HS-tk vector-producer cells. (Protocol #9206-019)

**Appendix D-30.** Dr. Albert D. Deisseroth of MD Anderson Cancer Center, Houston, Texas, can conduct gene transfer experiments on ten patients who have developed blast crisis or accelerated phase chronic myelogenous leukemia (CML). The retroviral vectors G1N and LNL6 which code for neomycin resistance will be used to transduce autologous peripheral blood and bone marrow cells that have been removed and stored at the time of cytogenetic remission or re-induction of chronic phase in Philadelphia chromosome positive CML patients. Following reinduction of the chronic phase of CML and preparative chemotherapy, patients will be infused with the transduced autologous cells.

This protocol is designed to determine the cause of relapse of CML. If polyclonal CML neomycin marked blastic cells appear at the time of relapse, their presence will indicate that relapse arises from the leukemic CML blast cells present in the autologous cells infused following chemotherapy. If residual systemic disease contributes to relapse, the neomycin resistance gene will not be detected in the CML leukemic blasts at the time of relapse.

This study will compare the relative contributions of the peripheral blood and bone marrow to generate hematopoietic recovery after bone marrow transplantation and evaluate purging and selection of peripheral blood or bone marrow as a source of stem cells for transplant. The percentage of neomycin resistant CML cells which are leukemic will be determined by PCR analysis and detection of bcr-abl mRNA. (Protocol #9206-020)

**Appendix D-31.** Dr. Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, can conduct gene transfer experiments on up to 48 patients with multiple myeloma, breast cancer, or chronic myelogenous leukemia. The retroviral vectors G1N and LNL6 will be used to transfer the neomycin resistance marker gene into autologous bone marrow and peripheral blood stem cells in the presence of growth factors to examine hematopoietic reconstitution after bone marrow transplantation. The efficiency of transduction of both short and long term autologous bone marrow reconstituting cells will be examined.

Autologous bone marrow and CD34+ peripheral blood stem cells will be enriched prior to transduction. Myeloma and CML patients will receive both autologous bone marrow and peripheral blood stem cell transplantation. These separate populations will be marked with both the G1N and LNL6 retroviral vectors. If short and long term marking experiments are successful, important information may be obtained regarding the biology of autologous reconstitution, the feasibility of retroviral gene transfer into hematopoietic cells, and the contribution of viable tumor cells within the autograft to disease relapse. (Protocol #9206-023/9206-024/9206-025)

**Appendix D-32.** Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with metastatic melanoma who are HLA-A2 positive and who have failed conventional therapy. This is a phase I study to examine whether allogeneic HLA-A2 matched melanoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-021)

**Appendix D-33.** Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with renal cell carcinoma who are HLA-A2 positive and who have failed conventional therapy. This Phase I study will examine whether allogeneic HLA-A2 matched renal cell carcinoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-022)

**Appendix D-34.** Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, can conduct experiments on twenty patients with metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer, or colon cancer who have failed standard therapy. Patients will receive multiple subcutaneous injections of autologous tumor cells combined with an autologous fibroblast cell line that has been transduced *in vitro* with the gene coding for Interleukin-4 (IL-4) to augment the *in vivo* antitumor effect. Patients will be monitored for antitumor effect by PCR analysis and multiple biopsy of the injection site. (Protocol #9209-033)

**Appendix D-35.** Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients  $\geq$  18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 10 patients per year will be enrolled in the studies over a period of four years. Patients will undergo autologous bone marrow transplantation with a selected population of Interleukin-3 (IL-3) or granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) peripheral blood repopulating cells (PBRC) that have been transduced with the gene coding for neomycin resistance (neo<sup>R</sup>) using the retroviral vector, LN. Patients will be continuously monitored for neo<sup>R</sup> to determine the relative contribution

of autologous PBRCs to long-term hematopoietic reconstitution. Demonstration of long-term contribution of autologous PBRC to hematopoiesis will enable the use of PBRC alone for autologous transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-027/9209-028)

**Appendix D-36.** Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients  $\geq 18$  years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 5 patients per year will be enrolled in the study over a period of four years. Patients will undergo allogeneic bone marrow transplant with granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) PBRC harvested from an identical twin that have been transduced with neo<sup>R</sup> using the retroviral vector, LN. Patients will be continuously monitored for neo<sup>R</sup> to determine the relative contribution of G-CSF stimulated allogeneic PBRCs to long-term bone marrow engraftment. Demonstration of long-term contribution of allogeneic PBRC to hematopoiesis will enable the use of PBRC alone for allogeneic transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-029)

**Appendix D-37.** Dr. Malcolm K. Brenner of St. Jude Children's Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, can conduct a multicenter uncontrolled human gene transfer experiment on 12 patients  $\leq 21$  years of age with Stage D Neuroblastoma in first or second marrow remission. Autologous bone marrow cells will be separated into two fractions, purged and unpurged. Each fraction will be transduced with the neo<sup>R</sup> gene by either LNL6 or G1Na. Patients will be monitored by the polymerase chain reaction (PCR) for the presence of neo<sup>R</sup>. The protocol is designed to evaluate the safety and efficacy of the Neuroblastoma Bone Marrow Purging System following high dose chemotherapy. (Protocol #9209-032)

**Appendix D-38.** Drs. Carolyn Keierleber and Ann Progulsk-Fox of the University of Florida, Gainesville, Florida, can conduct experiments involving the introduction of a gene coding for tetracycline resistance into *Porphyromonas gingivalis* at a physical containment level of Biosafety Level-2 (BL-2).

**Appendix D-39.** Dr. Scott M. Freeman of Tulane University Medical Center, New Orleans, Louisiana, can conduct experiments on patients with epithelial ovarian carcinoma who have clinical evidence of recurrent, progressive, or residual disease who have no other therapy available to prolong survival. Patients will be injected intraperitoneally with the irradiated PA-1 ovarian carcinoma cell line which has been transduced with the herpes simplex thymidine kinase (HSV-TK) gene. The patients will then receive ganciclovir therapy. Previous, data indicates that HSV-TK+ tumor cells exhibit a killing effect on HSV-TK- cells when exposed to ganciclovir therapy. Patients will be evaluated for safety and side effects of this treatment. (Protocol #9206-016)

**Appendix D-40.** Dr. Michael J. Welsh, Howard Hughes Medical Institute Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, may conduct experiments on 3 cystic fibrosis (CF) patients  $\geq 18$  years of age with mild to moderate disease. This Phase I study will determine the: (1) *in vivo* safety and efficacy of the administration of the replication-deficient type 2 adenovirus vector, Ad2/CFTR-1, to the nasal epithelium; (2) efficacy in correcting the CF chloride transport defect *in vivo*; and (3) effect of adenovirus vector dosage on safety and efficacy. (Protocol #9212-036)

**Appendix D-41.** Dr. Ronald G. Crystal, National Institutes of Health, Bethesda, Maryland, may conduct experiments on 10 cystic fibrosis (CF) patients  $\geq 21$  years of age. Patients will receive an initial administration of the replication-deficient type 5 adenovirus vector, AdCFTR, to their left nares. If no toxicity is observed from intranasal administration, patients will receive a single administration of AdCFTR to the respiratory epithelium of their left large bronchi. Five groups of patients (2 patients per group) will be studied based on increased dosage administration of AdCFTR. This study will determine the: (1) *in vivo* safety and efficacy of the administration of AdCFTR into the respiratory epithelium; (2) efficacy of the correction of the biologic abnormalities of CF in the respiratory epithelium; (3) duration of the biologic correction; (4) efficacy of the correction of the abnormal electrical potential difference of the airway epithelial sheet; (5) clinical parameters relevant to the disease process; and (6) if humoral immunity develops against AdCFTR sufficient to prevent repeat administration. (Protocol #9212-034)

**Appendix D-42.** Dr. Kenneth Culver, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John Van Gilder, University of Iowa, Iowa City, Iowa, may conduct experiments on 15 patients  $\geq 18$  years of age with recurrent malignant primary brain tumors or lung, melanoma, renal cell carcinoma, or breast carcinoma brain metastases who have failed standard therapy for their disease. Patient eligibility will be limited to those patients

who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies. The number of patients treated will be equally divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

Following surgical debulking, patients will receive a maximum of 3 intralesional injections of the G1TkSvNa vector-producing cell line (VPC) to induce regression of residual tumor cells by ganciclovir (GCV) therapy. Patients who demonstrate stable disease for a minimum of 6 months following this treatment will be eligible for additional VPC injections and subsequent GCV therapy. (Protocol #9303-037)

**Appendix D-43.** Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients  $\geq 1$  year and  $\leq 21$  years of age at the time of initial diagnosis of acute myelogenous leukemia (AML). The investigators will use the two retroviral vectors, LNL6 and G1Na, to determine the efficacy of the bone marrow purging techniques: 4-hydroxyperoxycyclophosphamide and interleukin-2 (IL-2) activation of endogenous cytotoxic effector cells, in preventing relapse from the reinfusion of autologous bone marrow cells. (Protocol #9303-039)

**Appendix D-44.** Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments of 35 patients  $\leq 21$  years of age who will be recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts for leukemia. In this Phase I dose escalation study, spontaneous lymphoblastoid cell lines will be established that express the same range of Epstein-Barr Virus (EBV) encoded proteins as the recipient. These EBV-specific cell lines will be transduced with LNL6 or G1Na and readministered at the time of bone marrow transplant. This study will determine: (1) survival and expansion of these EBV-specific cell lines *in vivo*, (2) the ability of these adoptively transferred cells to confer protection against EBV infection, and (3) appropriate dosage and administration schedules. (Protocol #9303-038)

**Appendix D-45.** Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., Gaithersburg, Maryland, may conduct experiments on 15 cystic fibrosis (CF) patients who have mild to moderate disease  $\geq 21$  years of age. The replication-deficient type 5 adenovirus vector, Av1CF2, will be administered to the nasal and lobar bronchial respiratory tract of patients. This study will demonstrate the: (1) expression of normal cystic fibrosis transmembrane conductance regulator (CFTR) mRNA *in vivo*, (2) synthesis of CFTR protein, and (3) correction of epithelial cell cAMP dependent  $Cl^-$  permeability. The pharmacokinetics of CFTR expression and ability to re-infect the respiratory tract with AvCF2 will be determined. Systemic and local immunologic consequences of Av1CF2 infection, the time of viral survival, and potential for recombination or complementation of the virus will be monitored. (Protocol #9303-041)

**Appendix D-46.** Dr. James M. Wilson of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct experiments on 20 adult patients with advanced cystic fibrosis lung disease. An isolated segment of the patients' lung will be transduced with the E1 deleted, replication-incompetent adenovirus vector, Ad.CB-CFTR using a bronchoscope for gene delivery. Ad.CB-CFTR contains the human gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Pulmonary biopsies will be obtained by bronchoscopy at 4 days, 6 weeks, and 3 months following treatment. Patients will be monitored for evidence of CFTR gene transfer and expression, immunological responses to CFTR or adenovirus proteins, and toxicity. (Protocol #9212-035)

**Appendix D-47.** Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina, may conduct experiments on 20 patients with disseminated malignant melanoma. Autologous tumor cells will be transduced with a retroviral vector, pHuy-IFN, that contains the gene encoding human  $\gamma$ -IFN. Following lethal irradiation, the transduced cells will be readministered to patients for the purpose of generating cytotoxic T cells that are tumor specific along with the up-regulation of Class I major histocompatibility antigens. Patients will be monitored for clinical regression of tumors and generation of tumor-specific cytotoxic T lymphocytes. (Protocol #9306-043)

**Appendix D-48.** Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of the Children's Hospital of Los Angeles, Los Angeles, California, may conduct experiments on 10 patients with Gaucher disease. CD34(+) hematopoietic stem cells will be isolated from bone marrow or from peripheral blood treated with granulocyte-colony stimulating factor. CD34(+) cells will

be transduced with a retrovirus vector, G1Gc, containing cDNA encoding human glucocerebrosidase and administered intravenously. Patients will be monitored for toxicity and glucocerebrosidase expression. (Protocol #9306-047)

**Appendix D-49.** Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 12 patients with AIDS to be divided into 4 experimental groups. CD4(+) lymphocytes will be isolated from peripheral blood and transduced with Rev M10, a transdominant inhibitory mutant of the *rev* gene of the human immunodeficiency virus (HIV). Transduction of the *rev* mutant will be mediated either by the retrovirus vector, PLJ-cREV M10, or by particle-mediated gene transfer of plasmid DNA. Patients will be monitored for survival of the transduced CD4(+) cells by polymerase chain reaction and whether Rev M10 can confer protection against HIV infection to CD4(+) cells. (Protocol #9306-049)

**Appendix D-50.** Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 24 patients with advanced cancer. Patients will undergo *in vivo* transduction with DNA/liposome complexes containing genes encoding the HLA-B7 histocompatibility antigen and beta-2 microglobulin in a non-viral plasmid. These DNA/liposome complexes will be administered either by intratumoral injection or catheter delivery. Patients will be monitored for enhanced immune responses against tumor cells, and safe and effective doses will be determined. (Protocol #9306-045)

**Appendix D-51.** Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct experiments on 5 patients with Gaucher disease. The CD34(+) hematopoietic stem cells will be isolated from peripheral blood and transduced *in vitro* with the retrovirus vector, N2-Sv-GC, encoding the glucocerebrosidase (GC) enzyme. Following reinfusion of the transduced cells, patients will be monitored by PCR analysis for GC expression in peripheral blood leukocytes. Patients currently receiving GC replacement therapy and who demonstrate clinical responsiveness will be withdrawn from exogenous GC therapy. Patients not previously treated with exogenous GC, will be monitored for clinical reversal of lipid storage symptoms. (Protocol #9306-046)

**Appendix D-52.** Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 12 HIV-infected patients who have a seronegative identical twin. CD4(+) and CD8(+) cells will be isolated from the seronegative twin and induced to polyclonal proliferation with anti-CD3 and interleukin-2. The enriched population of cells will be transduced with either LNL6 or G1Na, which contain the neo<sup>R</sup> gene. The transduced cells will be expanded in tissue culture and administered to the HIV-infected twin. Patients will be monitored for immune function and the presence of marked cells. (Protocol #9209-026)

**Appendix D-53.** Dr. Corey Raffel of the Children's Hospital Los Angeles, Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, may conduct experiments on 30 patients between 2 and 18 years of age with recurrent malignant astrocytoma. Fifteen patients will be accrued into this study initially. If at least one patient responds to therapy, an additional 14 patients will be treated. Patients with either surgically accessible or non-accessible tumors will be treated with the vector producing cell line (PA317) carrying the retrovirus vector, G1TkSvNa. This vector will transduce tumor cells *in vivo* with the *Herpes simplex* thymidine kinase (HS-tk) gene that renders the cells sensitive to killing by ganciclovir. Surgically accessible patients will undergo surgical debulking of their tumor followed by repeated administration of the HS-tk vector producer cells into the tumor bed. Children with unresectable tumors will undergo stereotaxic injection of vector producer cells into tumors. (Protocol #9306-050)

**Appendix D-54.** Dr. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, may conduct experiments on 15 HIV(+) asymptomatic patients. Patients will receive 3 monthly intramuscular injections of the retrovirus vector (N2IIIBenv) encoding the HIV-1 IIIB envelope protein. Patients will be monitored for acute toxicity, CD4 levels, HIV-specific CTL responses, and viral burdens. (Protocol #9306-048)

**Appendix D-55.** Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York, may conduct experiments on 20 patients with advanced breast, ovary, and brain cancer. CD34(+) hematopoietic stem cells will be isolated from bone marrow, transduced with the retrovirus vector, PHaMDR1/A, and readministered to patients. Patients will be monitored for the presence and expression of the MDR-1 gene. The investigators will determine whether MDR-1 expression increases following chemotherapy. (Protocol #9306-051)



**Appendix D-56.** Dr. Enzo Paoletti of Virogenetics Corporation, Troy, New York, may conduct experiments with poxvirus vectors NYVAC, ALVAC, and TROVAC at Biosafety Level 1.

**Appendix D-57.** Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, may conduct experiments on 9 patients (18 years old or greater) with cystic fibrosis to test for the safety and efficacy of an E1-deleted recombinant adenovirus containing the cystic fibrosis transmembrane conductance regulator (CFTR) cDNA, Ad.CB-CFTR. A single dose of  $10^8$ ,  $3 \times 10^9$  or  $10^{11}$  pfu/ml will be administered to the nasal cavity of 3 patients in each dose group. Patients will be monitored by nasal lavage and biopsy to assess safety and restoration of normal epithelial function. (Protocol #9303-042)

**Appendix D-58.** Dr. Joyce A. O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 18 patients (18-60 years old) with Stage IV breast cancer who have achieved a partial or complete response to induction chemotherapy. This study will determine the feasibility of obtaining engraftment of CD34(+) hematopoietic stem cells transduced by a retroviral vector, G1MD, and expressing a cDNA for the human multi-drug resistance-1 (MDR-1) gene following high dose chemotherapy, and whether the transduced MDR-1 gene confers drug resistance to hematopoietic cells and functions as an *in vivo* dominant selectable marker. Patients will be monitored for evidence of myeloprotection and presence of the transduced MDR-1 gene." (Protocol #9309-054)

**Appendix D-59.** Drs. Larry E. Kun, R. A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients (3-21 years old) with progressive or recurrent malignant supratentorial tumors resistant to standard therapies. Mouse cells producing the retroviral vector containing the herpes simplex thymidine kinase gene (G1TKSVNa) will be instilled into the tumor areas via multiple stereotactically placed cannulas. Patients will be treated with ganciclovir to eliminate cells expressing the transduced gene. Patients will be monitored for central nervous system, hematologic, renal or other toxicities, and for anti-tumor responses by magnetic resonance imaging studies. (Protocol #9309-055)

**Appendix D-60.** The physical containment level may be reduced from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system of Life Technologies, Inc., Gaithersburg, Maryland.

**Appendix D-61.** Dr. Albert B. Deisseroth of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 10 patients ( $\geq 16$  to  $\leq 60$  years of age) with chronic lymphocytic leukemia. Autologous peripheral blood and bone marrow cells will be removed from patients following chemotherapy and marked by transduction with two distinguishable retroviral vectors, G1Na and LNL6, containing the neomycin resistance gene. The gene marked cells will be reinfused into patients to determine the efficiency of bone marrow purging and the origin of relapse following autologous bone marrow transplantation. (Protocol #9209-030)

**Appendix D-62.** Dr. Jonathan Simons of the Johns Hopkins Oncology Center, Baltimore, Maryland, may conduct experiments on 26 patients ( $\geq 18$  years of age) with metastatic renal cell carcinoma to evaluate the safety and tolerability of intradermally injected autologous irradiated tumor cells transduced with the retrovirus vector, MFG, containing the human granulocyte-macrophage colony stimulating factor gene. Acute and long-term clinical toxicities and *in vitro* and *in vivo* induction of specific anti-tumor immune responses will be monitored. (Protocol #9303-040)

**Appendix D-63.** Dr. Albert B. Deisseroth of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 20 patients ( $\geq 18$  and  $\leq 60$  years old) with ovarian cancer. A murine viral vector was constructed from the third generation of L series retroviruses with the insert of the human multi-drug resistance-1 (MDR-1) transduced gene. The investigators will assess the safety and feasibility of administering CD34 (+) autologous peripheral blood and bone marrow cells. Patients will be monitored for the presence of the MDR-1 gene and for the effect of gene transfer on hematopoietic function following the transplantation. (Protocol #9306-044)

**Appendix D-64.** Dr. Joseph Ilan of the Case Western Reserve University School of Medicine and University Hospital of Cleveland, Cleveland, Ohio, may conduct experiments on 12 patients ( $\geq 18$  years of age) with advanced brain cancer. Human malignant glioma tumor cells will be cultured, transfected with Epstein-Barr virus (EBV)-based vector, anti-Insulin growth factor-I, lethally irradiated, and injected subcutaneously into

patients in an attempt to express antisense Insulin growth factor-1. Patients will be monitored for toxicity and immunologic responses to the vector. (Protocol #9306-052)

**Appendix D-65.** Drs. James S. Economou and John Glaspy of the University of California, Los Angeles, California, may conduct experiments on 30 patients ( $\geq 18$  to  $\leq 70$  years of age) with metastatic melanoma. A human melanoma cell line (M-24) will be transduced with the retroviral vector, G1NaCvi2, expressing the human interleukin-2 (IL-2) gene. The IL-2 producing cells will be mixed with the patient's autologous tumor cells, irradiated, and injected subcutaneously in an attempt to enhance the tumor-specific immunologic response. Patients will be monitored for toxicity, *in vitro* and *in vivo* immunologic responses, and clinical anti-tumor effects. (Protocol #9309-058)

**Appendix D-66.** Drs. Peter Cassileth, Eckhard R. Podack, and Kasi Sridhar of the University of Miami, and Niramol Savaraj of the Miami Veterans Administration Hospital, Miami, Florida, may conduct experiments on 12 patients ( $\geq 18$  years of age) with limited stage small cell lung cancer. Autologous tumor cells will be removed, expanded in culture, and transduced by lipofection with the BMG-Neo-hIL2 vector (derived from bovine papilloma virus). The objective of this protocol is to demonstrate the safety and efficacy of administering IL-2 transduced autologous tumor cells in an attempt to stimulate a tumor-specific cytotoxic T lymphocyte (CTL) response, and to determine the quantity and characteristics of the CTL that have been generated. (Protocol #9309-053)

**Appendix D-67.** Drs. Edward H. Oldfield and Zvi Ram of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 20 patients ( $\geq 18$  years of age) with leptomeningeal carcinomatosis. The patients will receive intraventricular or subarachnoid injection of murine vector producing cells containing the retroviral vector, G1Tk1SvNa. Tumor cells expressing the herpes simplex thymidine kinase gene will be rendered sensitive to killing by subsequent administration of ganciclovir. Patients will be monitored for safety and anti-tumor response by magnetic resonance imaging (MRI) and cerebral spinal fluid cytological analysis. (Protocol #9312-059)

**Appendix D-68.** Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Chicago, Illinois, may conduct experiments on 12 subjects who differ in at least 3 out of 6 alleles at the Class I histocompatibility locus ( $\geq 18$  years of age) with Stage IV malignant melanoma. The subjects will be immunized with a lethally irradiated allogeneic human melanoma cell line transduced with the human interleukin-2 expressing retroviral vector, pZipNeoSv-IL-2. Subjects will be monitored for toxicity, induction of B and T cell antitumor responses *in vitro* and *in vivo*, and any clinical evidence of antitumor effect. (Protocol #9309-056)

**Appendix D-69A.** Dr. Michael J. Welsh of the Howard Hughes Medical Institute, Iowa City, Iowa, may conduct experiments on 20 patients ( $\geq 18$  years of age) with cystic fibrosis. The investigator will administer increasing doses of either of the two adenovirus vectors, AD2/CFTR-1 or AD2-ORF6/PGK-CFTR, to the nasal epithelium of 10 patients (1 nostril) or maxillary sinus epithelium of 10 patients (1 maxillary sinus). Patients will be isolated for a period of 24 hours following vector administration; however, if 1 patient demonstrates secreted virus at 24 hours, the investigator will notify the Recombinant DNA Advisory Committee for reconsideration of the isolated period. Patients will be assessed for the safety and efficacy of multiply administration of adenovirus vectors encoding the cystic fibrosis transmembrane conductance regulator (CFTR) gene. (Protocol #9312-067)

**Appendix D-69B.** Dr. Richard Haubrich of the University of California at San Diego Treatment Center, San Diego, California, may conduct experiments on 25 human immunodeficiency virus (HIV)-infected, seropositive, asymptomatic subjects ( $\geq 18$  to  $\leq 65$  years of age). Subjects will receive 3 monthly intramuscular injections of the retroviral vector, N2/IIIB *env/rev*, which encodes for HIV-1 IIIB *env/rev* proteins. The objective of the study is to induce HIV-1- specific CD8(+) cytotoxic T lymphocyte and antibody responses in order to eliminate HIV-infected cells and residual virus. This Phase I/II study will evaluate acute toxicity, identify long-term treatment effects, and evaluate the disease progression. (Protocol #9312-062)

**Appendix D-70.** Dr. Mario Sznol of the National Institutes of Health, Frederick, Maryland, may conduct experiments on 50 subjects ( $\geq 18$  years of age) with advanced stage melanoma. Subjects will receive subcutaneous injections of lethally irradiated allogeneic melanoma cells that have been transduced by lipofection with the plasmid DNA vector, CMV-B7, derived from bovine papilloma virus to express the human B7 antigen. The B7 antigen, which binds to the CD28 receptor of T cells, will serve as a co-stimulatory signal to elicit an antitumor immune response. Subjects will be monitored for induction of cytotoxic T lymphocyte, antitumor responses *in vitro* and *in vivo* and any clinical evidence of antitumor effect. (Protocol #9312-063)

**Appendix D-71.** Dr. Joseph Rubin of the Mayo Clinic, Rochester, Minnesota, may conduct experiments on 15 subjects with hepatic metastases from advanced colorectal cancer ( $\geq 18$  years of age). Subjects will receive intratumoral hepatic injections of the plasmid DNA/lipid complex, pHLA-B7/ $\beta$ -2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and  $\beta$ -2 microglobulin. Subjects must be HLA-B7 negative. The objective of this study is to determine a safe and effective dose of the DNA/lipid complex. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9312-064)

**Appendix D-72.** Dr. Nicholas J. Vogelzang of the University of Chicago Medical Center, Chicago, Illinois, may conduct experiments on 15 subjects with metastatic renal cell carcinoma  $\geq 18$  years of age. Subjects will receive intratumoral injections of the plasmid DNA/liposome vector pHLA-B7/ $\beta$ -2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and  $\beta$ -2 microglobulin. Subjects must be HLA-B7 negative. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9403-071)

**Appendix D-73.** Dr. Evan M. Hersh of the Arizona Cancer Center and Drs. Emmanuel Akporiaye, David Harris, Alison T. Stopeck, Evan C. Unger, and James A. Warneke of the University of Arizona, Tucson, Arizona, may conduct experiments on 15 subjects with metastatic malignant melanoma  $\geq 18$  years of age. Subjects will receive intratumoral injections of the plasmid DNA/liposome vector, pHLA-B7/ $\beta$ -2 microglobulin, expressing a heterodimeric cell surface protein consisting of the HLA-B7 histocompatibility antigen and  $\beta$ -2 microglobulin. Subjects must be HLA-B7 negative. Subjects will be monitored for antigen-specific immune responses and *in vivo* HLA-B7 expression. (Protocol #9403-072)

**Appendix D-74.** Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas, may conduct gene marking experiments on 9 subjects with ovarian carcinoma or peritoneal carcinomatosis ( $\geq 16$  years of age). Autologous CD3(+)/CD8(+) tumor infiltrating lymphocyte derived T cells will be transduced with the retroviral vector G1Na that encodes for neo<sup>R</sup>. Subjects will receive intraperitoneal administration of bulk expanded transduced and nontransduced T cells and recombinant interleukin-2. Previously documented tumor sites and normal tissues will be monitored for neo<sup>R</sup> and the proportion of CD3(+)/CD8(+) T cells will be determined. (Protocol #9406-075)

**Appendix D-75.** Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct gene marking experiments on 20 subjects undergoing autologous bone marrow transplantation for therapy of leukemia or solid tumor ( $< 21$  years of age). The distinguishable retroviral vectors, LNL6 and G1Na (both encoding neo<sup>R</sup>), will be used to determine the rate of reconstitution of untreated versus cytokine expanded CD34(+) selected autologous bone marrow cells. (Protocol #9406-076)

**Appendix D-76.** Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas, may conduct experiments on 10 fully evaluable subjects (maximum of 20 entered) with Stage III or IV breast cancer ( $\geq 18$  and  $\leq 60$  years of age). Subjects will receive autologous CD34(+) peripheral blood cells that have been transduced with the retroviral vector, pVM DR-1, which encodes the multi-drug resistance gene. The objective of this study is to evaluate the safety and feasibility of transducing early hematopoietic progenitor cells with pVM DR-1 and to determine *in vivo* selection of chemotherapy resistant hematopoietic cells. (Protocol #9406-077)

**Appendix D-77.** Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients with Fanconi anemia ( $\geq 5$  years of age). Subjects will receive autologous CD34(+) cells that have been transduced with the retroviral vector, FACC, which encodes the normal Fanconi anemia complementation group C gene. The objective of this study is to determine whether autologous FACC transduced hematopoietic progenitor cells can be safely administered to subjects, the extent of engraftment, and correction of cell phenotype. (Protocol #9406-078)

**Appendix D-78.** Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, may conduct experiments on 15 subjects with recurrent residual glioblastoma multi-forme ( $\geq 18$  years of age). Subjects will receive subcutaneous injections of autologous tumor cells that have been lethally irradiated and transduced with the retroviral vector, G1NaCvi2.23, which encodes for interleukin-2. Subjects will be monitored *in vitro* for cellular and humoral antitumor responses and *in vivo* for antitumor activity. (Protocol #9406-080)

**Appendix D-79.** Dr. Alfred E. Chang of the University of Michigan Medical Center, Ann Arbor Michigan, may conduct gene marking experiments on 15 subjects with metastatic melanoma ( $\geq 18$  years of age). Subjects will undergo adoptive immunotherapy of anti-CD3/interleukin-2 activated lymph node cells that have been primed *in vivo* with tumor cells that have been transduced with the retrovirus vector, GBAH4, encoding the gene for interleukin-4. The investigator will evaluate the antitumor efficacy and *in vivo* immunological reactivity in subjects receiving adoptively transferred T cells, and the *in vitro* immunological reactivities of the activated T cells that might correlate with their *in vivo* antitumor function. (Protocol #9312-065)

**Appendix D-80.** Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct gene marking experiments on 40 HIV(+) subjects ( $\geq 18$  years of age). The investigator may also enter an additional number of subjects (to be determined by the investigator) who will receive a single administration of  $1 \times 10^7$  HIV-specific CD8(+) cells. The investigator will: (1) Assess the safety and tolerance of the adoptive transfer of anti-HIV cytotoxic, syngeneic, CD8(+) peripheral blood lymphocytes that have been transduced with the retrovirus vector, *rkat4svgF3e-*, that encodes for a universal chimeric T cell receptor. (2) Determine the longevity of the genetically marked CD8(+) lymphocytes in the subject's peripheral blood. (Protocol #9403-069)

**Appendix D-81.** Dr. Joseph Rosenblatt of the University of California, Los Angeles, California, and Dr. Robert Seeger of Children's Hospital, Los Angeles, California, may conduct gene transfer experiments on 18 subjects with neuroblastoma ( $\leq 21$  years of age). Patients at high risk of relapse with minimal or no detectable disease following myeloablative therapy and autologous bone marrow transplantation, or patients with progressive or persistent disease despite conventional therapy will be serially immunized with autologous or allogeneic neuroblastoma cells transduced to express  $\gamma$  interferon. Cells will be transduced with the retroviral vector, pH $\gamma$ -IFN, encoding the human gene for  $\gamma$  interferon and lethally irradiated prior to use as an immunogen. The objectives of the study are: (1) to determine the maximum tolerable dose of transduced cells; (2) to determine the local, regional, and systemic toxicities of injected cells; and (3) to determine the antitumor response *in vivo* as measured by standard clinical tests and immunocytologic evaluation of marrow metastases. (Protocol #9403-068)

**Appendix D-82.** Dr. Kenneth L. Brigham of Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 subjects ( $\leq 21$  years of age) in two different patient protocols (5 for each protocol). Both protocols will use the same DNA/liposome preparations to deliver a plasmid DNA construct, pCMV4-AAT, encoding human alpha-1 antitrypsin gene driven by a cytomegalovirus promoter. In patients scheduled for elective pulmonary resection, the DNA/liposome complexes will be instilled through a fiber optic bronchoscope into a subsegment of the lung. Tissues of the lung will be obtained at the time of surgery. Transgene expression will be assessed by immunohistochemistry, *in situ* hybridization, and Western and Northern blot analyses. The effect of DNA/liposome complex administration on the histological appearance of the lung will also be evaluated. In patients with alpha-1 antitrypsin deficiency, the DNA/liposome complexes will be instilled into the nostril. Transgene expression will be determined in cells obtained by nasal lavage and nasal scraping, and the time course of transgene expression will be measured. The secretion of the alpha-1 antitrypsin protein in nasal fluid will be determined. Histological appearance of nasal mucosa will also be examined. The study will assess safety and feasibility of gene delivery to the human respiratory tract. (Protocol #9403-070)

**Appendix D-83.** Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 20 subjects with refractory or recurrent metastatic breast cancer ( $\geq 18$  years of age). Autologous breast cancer cells will be transduced with the DNA/liposome complex, pMP6-IL2, containing a plasmid DNA vector derived from adeno-associated virus (AAV) that expresses the gene for human interleukin-2. Subjects will receive 4 subcutaneous injections of lethally irradiated tumor cells transduced with the DNA/liposome complex prior to injection. The objective of this study is to: (1) evaluate the safety and toxicity of the treatment, (2) determine the immunological effects, (3) determine the duration of clinical responses, and (4) measure patient survival. (Protocol #9409-086)

**Appendix D-84.** Drs. Flossie Wong-Staal, Eric Poeschla, and David Looney of the University of California at San Diego, La Jolla, California, may conduct gene transfer experiments on 6 subjects ( $\geq 18$  and  $\leq 65$  years of age) infected with human immunodeficiency virus-1 (HIV-1). Autologous CD4(+) T lymphocytes will be transduced *ex vivo* with the retroviral vector, pMJT, expressing a hairpin ribozyme that cleaves the HIV-1 RNA in the 5' leader sequence. The transduced cells will be expanded and reinfused into the patients. The objectives of the study are: (1) to evaluate safety of reinfusing the transduced lymphocytes, (2) to compare (*in vivo*) the kinetics and survival of ribozyme-transduced cells with that of cells transduced with a control vector, (3) to determine *in vivo* expression of the ribozyme sequences in transduced lymphocytes, (4) to determine whether host immune responses directed against the transduced cells will occur *in vivo*, and (5) to obtain preliminary data on the effects of ribozyme gene therapy on *in vivo* HIV mRNA expression, viral burden and CD4(+) lymphocyte levels. (Protocol #9309-057)

**Appendix D-85.** Dr. Friedrich Schuening of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 10 subjects ( $\geq 18$  years of age) with Type I Gaucher's disease. The peripheral blood repopulating cells (mobilized by patient pretreatment with recombinant granulocyte colony-stimulating factor) will be harvested and CD34(+) cells selected. CD34(+) cells will be transduced *ex vivo* with the retroviral vector, LgGC, that encodes human glucocerebrosidase cDNA. Following transduction, the transduced cells will be infused into the patient without myeloablative treatment. The primary endpoint of this study is to examine the safety of infusing CD34(+) cells transduced with the human glucocerebrosidase cDNA. Patients will be monitored for persistence and expression of the glucocerebrosidase gene in hematopoietic cells. (Protocol #9312-061)

**Appendix D-86.** Dr. Terence R. Flotte of the Johns Hopkins Children's Center, Baltimore, Maryland, may conduct gene transfer experiments on 16 subjects ( $\geq 18$  years of age) with mild cystic fibrosis (CF). An adeno-associated virus (AAV) derived vector, encoding cystic fibrosis transmembrane conductance regulator (CFTR) gene, (tgAAVCF), will be administered to nasal (direct) and airway (bronchoscope) epithelial cells. This is a dose escalation study involving 8 cohorts. Each subject will receive both intranasal and bronchial administration of the adenoviral vector at 4 escalating doses. Nasal doses will range between  $1 \times 10^6$  and  $1 \times 10^9$  pfu. Lung administration will range between  $1 \times 10^7$  and  $1 \times 10^{10}$  pfu. The primary goal of the study is to assess the safety of vector administration. Respiratory and nasal epithelial cells will be evaluated for gene transfer, expression, and physiologic correction. (Protocol #9409-083)

**Appendix D-87.** Drs. Jeffrey M. Isner and Kenneth Walsh of St. Elizabeth's Medical Center, Tufts University, Boston, Massachusetts, may conduct gene transfer experiments on 12 subjects ( $\geq 40$  years of age) with peripheral artery disease (PAD). A plasmid DNA vector, phVEGF165, encoding the human gene for vascular endothelial growth factor (VEGF) will be used to express VEGF to induce collateral neovascularization. Percutaneous arterial gene transfer will be achieved using an angioplasty catheter with a hydrogel coated balloon to deliver the plasmid DNA vector to the artery. The objectives of the study are: (1) to determine the efficacy of arterial gene therapy to relieve rest pain and/or heal ischemic ulcers of the lower extremities in patients with PAD; and (2) to document the safety of the phVEGF arterial gene therapy for therapeutic angiogenesis. Subjects will undergo anatomic and physiologic examination to determine the extent of collateral artery development following phVEGF arterial gene therapy. (Protocol #9409-088)

**Appendix D-88A.** Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 26 patients ( $\geq 15$  years of age) with cystic fibrosis (CF). A replication deficient recombinant adenovirus vector will be used to transduce epithelial cells of the large bronchi with the E1/E3 deleted type 5 adenovirus vector, Ad<sub>GV</sub>CFTR.10, which encodes the human cystic fibrosis transmembrane conductance regulator (CFTR) gene. The objective of this study is to define the safety and pharmacodynamics of CFTR gene expression in airway epithelial cells following single administration of escalating doses to the vector. If single administration is determined to be safe, subjects will undergo repeat administration to localized areas of the bronchi. (Protocol #9409-085)

**Appendix D-88B.** Drs. Eric J. Sorscher and James L. Logan of the University of Alabama, Birmingham, Alabama, may conduct gene transfer experiments on 9 subjects ( $\geq 18$  years of age) with cystic fibrosis (CF). The normal human cystic fibrosis transmembrane conductance regulator (CFTR) gene will be expressed by a plasmid DNA vector, pKCTR, driven by the simian virus-40 (SV40) early gene promoter. The CFTR DNA construct will be delivered by cationic liposome-based gene transfer to nasal epithelial cells. The objectives of the study are to: (1) evaluate the safety of lipid-mediated gene transfer to nasal epithelial cells (including local inflammation and mucosal tissue); and (2) evaluate efficacy as determined by correction of the chloride ion transport defect, and wild-type CFTR mRNA and protein expression. (Protocol #9312-066)

**Appendix D-89.** Dr. Steven M. Albelda of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 12 subjects with advanced mesothelioma. The adenovirus vector encoding the *Herpes simplex* virus thymidine kinase (HSV-TK) gene, H5.020RSVTK, will be administered through a chest tube to the pleural cavity. Tumor biopsies will be assayed for gene transfer and expression. Subjects will be monitored for immunological responses to the adenovirus vector. Ganciclovir will be administered intravenously 14 days following vector administration. The primary objective of this Phase I study is to evaluate the safety of direct adenovirus vector gene delivery to the pleural cavity of patients with malignant melanoma. (Protocol #9409-090)

**Appendix D-90.** Drs. Jeffrey Holt and Carlos B. Arteaga of the Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 female patients (over 18 years of age) with metastatic breast cancer. Patient effusions from pleura or peritoneum will be drained and the fluid will be replaced with supernatant containing the retroviral vectors, XM6:antimyc or XM6:antifos, which express *c-myc* and *c-fos* antisense sequences, respectively, under the control of a mouse mammary tumor virus promoter. The objectives of this study are to: (1) assess uptake and expression of the vector sequences in breast cancer cells present in pleural and peritoneal fluids, and determine if this expression is tumor specific, (2) assess the safety of localized administration of antisense retroviruses, and (3) monitor subjects for clinical evidence of antitumor response. (Protocol #9409-084)

**Appendix D-91.** Dr. Jack A. Roth of MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 14 non-small cell lung cancer subjects ( $\geq 18$  and  $\leq 80$  years of age) who have failed conventional therapy and who have bronchial obstruction. LNSX-based retroviral vectors containing the  $\beta$ -actin promoter will be used to express: (1) the antisense RNA of the *K-ras* oncogene (LN-K-*rasB*), and (2) the wildtype *p53* tumor suppressor gene (LN*p53B*). Tumor biopsies will be obtained to characterized *K-ras* and *p53* mutations. Relative to their specific mutation, subjects will undergo partial endoscopic resection of the tumor bed followed by bronchoscopic administration of the appropriate retrovirus construct. The objective of this study is to evaluate the safety and efficacy of intralesional administration of LN-K-*rasB* and LN*p53* retrovirus constructs. (Protocol #9403-031)

**Appendix D-92.** Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, may conduct gene transfer experiments on 12 subjects ( $\geq 18$  years of age) with metastatic colon carcinoma. The autologous skin fibroblasts will be transduced with the retroviral vector, LNCX/IL-2, which encodes the gene for human interleukin-2 (IL-2). In this dose-escalation study, subjects will receive subcutaneous injections of lethally irradiated autologous tumor cells. The objectives of the study are to: (1) evaluate the safety of subcutaneous administration of LNCX/IL-2 transduced fibroblasts, (2) determine *in vivo* antitumor activity, and (3) monitor cellular and humoral antitumor responses. (Protocol #9312-060)

**Appendix D-93.** Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct gene transfer experiments on 18 subjects ( $\geq 18$  years of age) with advanced melanoma, 6 with T-cell lymphoma, breast cancer, or head and neck cancer. Subjects should have accessible cutaneous tumors, and have failed standard therapy. Over 4 weeks, subjects will receive a total of 4 intratumoral injections of autologous fibroblasts transduced with the retrovirus vector, TFG-hIL-12-Neo. This vector, which consists of the murine MFG backbone, expresses both the p35 and p40 subunits of interleukin-12 (IL-12) and the *neo*<sup>R</sup> selection marker. The objectives of the study are to: (1) define the local and systemic toxicity associated with peritumoral injections of gene-modified fibroblasts, (2) examine the local and systemic immunomodulatory effects of these injections, and (3) evaluate clinical antitumor efficacy. (Protocol #9406-081)

**Appendix D-94.** Drs. Evan Hersh, Emmanuel Akporiaye, David Harris, Alison Stopeck, Evan Unger, James Warneke, of the Arizona Cancer Center, Tucson, Arizona, may conduct gene transfer experiments on 25 subjects ( $\geq 18$  years of age) with solid malignant tumors or lymphomas. A plasmid DNA/lipid complex designated as VCL-1102 (IL-2 Plasmid DNA/DMRIE/DOPE) will be used to transduce the human gene for interleukin-2 (IL-2). Patients with advanced cancer who have failed conventional therapy will undergo a procedure in which VCL-1102 is injected directly into the tumor mass to induce tumor-specific immunity. The objectives of the study are to: (1) determine safety and toxicity associated with escalating doses of VCL-1102; (2) confirm IL-2 expression in target cells; (3) determine biological activity and pharmacokinetics; and (4) determine whether IL-2 expression stimulates tumor regression in subjects with metastatic malignancies. (Protocol #9412-095)

**Appendix D-95.** Drs. Richard Morgan and Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiments on 48 human immunodeficiency virus (HIV) seropositive subjects ( $\geq 18$  years of age). This Phase I/II study involves identical twins (one HIV seropositive and the other HIV seronegative). CD4(+) T cells will be enriched following apheresis of the HIV seronegative twin, induced to polyclonal proliferation with anti-CD3 and recombinant IL-2, transduced with either the LNL6/Neo<sup>R</sup> or G1Na/Neo<sup>R</sup>, and transduced with up to 2 additional retroviral vectors (G1RevTdsN and/or GCRTdsN(TAR)) containing potentially therapeutic genes (antisense TAR and/or transdominant Rev). These T cell populations will be expanded 10 to 1,000 fold in culture for 1 to 2 weeks and reinfused into the HIV seropositive twin. Subjects will receive up to 4 cycles of treatment using identical or different combinations of control and anti-HIV retrovirus vectors. The relative survival of these transduced T cell populations will be monitored by vector-specific polymerase chain reaction, while the subjects' functional immune status is monitored by standard *in vitro* and *in vivo* assays. (Protocol #9503-103)

**Appendix D-96.** Dr. Harry L. Malech of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiments on 2 subjects  $\geq 18$  years of age (with or without concurrent serious infection), and 3 subjects  $\geq 18$  years of age (with or without concurrent serious infection) or minors 13-17 years of age who have concurrent serious infection who have chronic granulomatous disease (CGD). CGD is an inherited immune deficiency disorder in which blood neutrophils and monocytes fail to produce antimicrobial oxidants (p47<sup>phox</sup> mutation) resulting in recurrent life-threatening infections. Subjects will undergo CD34(+) mobilization with granulocyte colony stimulating factor (G-CSF). These CD34(+) cells will be transduced with the retrovirus vector, MFG-S-p47<sup>phox</sup>, which encodes the gene for normal p47<sup>phox</sup>. The objectives of this study are to: (1) determine the safety of administering MFG-S-p47<sup>phox</sup> transduced CD34(+) cells, and (2) demonstrate increased functional oxidase activity in circulating neutrophils. (Protocol #9503-104)

**Appendix D-97.** Drs. Chris Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct gene transfer experiments on 6 subjects ( $\geq 18$  and  $\leq 76$  years of age) with rheumatoid arthritis. Rheumatoid arthritis is a chronic, progressive disease thought to be of autoimmune origin. A gene encoding an interleukin-1 receptor antagonist protein (IRAP) will be delivered to the rheumatoid metacarpal-phalangeal joints to determine the autoimmune reactions can be interrupted. The vector construct, DFG-IRAP, is based on the MFG murine retrovirus vector backbone, and encodes the human IRAP gene. Synovial fibroblasts will be generated from the rheumatoid arthritic joint tissue obtained from patients who are scheduled to undergo surgery. The fibroblasts will be transduced with the DFG-IRAP vector, and the transduced cells injected into the synovial space. The synovial fluid and joint material will be collected 7 days later to determine the presence and location of the transduced synovial fibroblasts and the level of IRAP in the joint fluid. (Protocol 9406-074)

**Appendix D-98.** Dr. R. Scott McIvor of the University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on 2 children with purine nucleoside phosphorylase (PNP) deficiency. PNP deficiency results in severe T-cell immunodeficiency, an autosomal recessive inherited disease which is usually fatal in the first decade of life. Autologous peripheral blood lymphocytes will be cultured in an artificial capillary cartridge in the presence of anti-CD3 monoclonal antibody and interleukin-2 and transduced with the retroviral vector, LPNSN-2, encoding human PNP. Subjects will undergo bimonthly intravenous administration of transduced T cells for a maximum of 1 year. The objectives of the study are to determine: (1) the safety of intravenous administration of transduced T cells in children with PNP deficiency, (2) the efficiency of PNP gene transfer and duration of gene expression *in vivo*, and (3) the effect of PNP gene transfer on immune function. (Protocol #9506-110)

**Appendix D-99.** Drs. Nikhil C. Munshi and Bart Barlogie of the University of Arkansas School for Medical Sciences, Little Rock, Arkansas, may conduct gene transfer experiments on 21 subjects (>18 and <65 years of age) with relapsed or persistent multiple myeloma who are undergoing T cell depleted allogeneic bone marrow transplantation. Donor peripheral blood lymphocytes will be cultured in vitro with interleukin-2 and anti-CD3 monoclonal antibody. T cell depleted lymphocytes will be transduced with the retroviral construct, G1Tk1SvNa.7, which encodes the Herpes simplex virus thymidine kinase (HSV-TK) gene. The transduced cells will be reinfused. In this dose escalation study, 3 subjects will undergo cell-mediated gene transfer per cohort (maximum of 5 cohorts) until Grade III or IV Graft versus Host Disease (GVHD) is observed. A maximum of 6 additional patients may be entered at that maximum tolerated dose. The objectives of this study are to determine the: (1) safety of transduced donor cell infusions, (2) effectiveness of donor cell infusions in decreasing the effects of severe GVHD, (3) effectiveness of donor cell infusions in prolonging multiple myeloma remission, and (4) effectiveness of ganciclovir in eliminating donor cells for the purpose of preventing the depletion of erythrocytes. (Protocol #9506-107)

**Appendix D-100.** Dr. Wayne A. Marasco of Dana-Farber Cancer Institute, Boston, Massachusetts, may conduct gene transfer experiments on 6 subjects ( $\geq 18$  and  $\leq 65$  years of age) with human immunodeficiency virus type-1 (HIV-1). Autologous lymphocytes from asymptomatic subjects will be transduced *ex vivo* with a retroviral vector, LNCs105, encoding the sFv105 antibody specific for the HIV-1 envelope protein. An identical aliquot will be simultaneously transduced with a control retroviral vector lacking the sFv105 cassette. Transduced cells will be reinfused into patients and the differential survival of both populations of CD4+ lymphocytes compared. The objective of the study is to determine whether the intracellular expression of a human single chain antibody against HIV-1 envelope glycoprotein gp160 that blocks gp160 processing and the production of infectious virions can safely prolong the survival of CD4(+) lymphocytes in HIV-1-infected subjects. (Protocol #9506-111)

**Appendix D-101.** Dr. Henry Dorkin of the New England Medical Center, Boston, Massachusetts, and Dr. Allen Lapey of Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, propose to conduct gene transfer experiments on 16 subjects ( $\geq 18$  years of age). An E1/partial E4-deleted, replication-deficient, type 2 adenovirus vector, AD2/CFTR-2, will be used to deliver the human cystic fibrosis transmembrane conductance regulator (CFTR) gene by aerosol administration (nebulization) to the lung of CF patients. Aerosol administration will be initiated only after initial safety data has been obtained from the lobar administration protocol (#9409-091). This is a single administration dose-escalation study in which subjects will receive between  $8 \times 10^6$  and  $2.5 \times 10^{10}$  pfu. Subjects will be assessed for evidence of adverse, systemic, immune, inflammatory, or respiratory effects in response to AD2/CFTR-2. Subjects will be monitored for virus shedding and transgene expression. Health care workers present in the facility will be required to sign an Informed Consent document regarding the possibility of virus transmission. (Protocol #9412-074)

**Appendix D-102.** Drs. Charles J. Link and Donald Moorman of the Human Gene Therapy Research Institute, Des Moines, Iowa, may conduct gene transfer experiments on 24 female subjects ( $\geq 18$  years of age) with refractory or recurrent ovarian cancer. Subjects will undergo intraperitoneal delivery (via Tenkhoff catheter) of the vector producing cells (VPC), PA317/LTKOSN.2. These VPC express the *Herpes simplex* virus thymidine kinase (HSV-TK) gene which confers sensitivity to killing by the antiviral drug, ganciclovir (GCV). The LTKOSN.2 retrovirus vector is based on the LXS backbone. Two weeks following intraperitoneal delivery of the VPC, subjects will receive 5 mg/kg intravenous GCV twice daily for 14 days. Subjects will receive between  $1 \times 10^5$  and  $1 \times 10^8$  VPC/kg in this dose escalation study. Subjects will be evaluated by X-ray and peritoneoscopy of the abdomen for evidence of clinical response. The objectives of this study are to determine the safety of intraperitoneal VPC administration. (Protocol #9503-100)

**Appendix D-103.** Dr. David T. Curiel of the University of Alabama, Birmingham, Alabama, may conduct gene transfer experiment of 15 subjects ( $\geq 18$  years of age) with metastatic colorectal cancer. Subjects will receive intramuscular injection of the polynucleotide vaccine, pGT63, which is a plasmid DNA vector expressing carcinoembryonic antigen (CEA) and hepatitis B surface antigen (HBsAg). The objectives of the study are to: (1) characterize the immune response to CEA and HBsAg following a single intramuscular injection and following 3 consecutive intramuscular injections, and (2) determine the safety of intramuscular injection of the plasmid DNA vector at doses ranging between 0.1 to 1.0 milligrams (single dose) and 0.9 to 3.0 milligrams (total multidose). (Protocol #9506-073)



**Appendix D-104.** Dr. Chester B. Whitley of the University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on two adult subjects (18 years of age or older) with mild Hunter syndrome (Mucopolysaccharidosis Type II). The autologous peripheral blood lymphocytes will be transduced *ex vivo* with the retroviral vector, L2SN, encoding the human cDNA for iduronate-2-sulfatase (IDS). The transduced lymphocytes will be reinfused into the patients on a monthly basis. The study will determine the frequency of peripheral blood lymphocyte transduction and the half-life of the infused cells. Evaluation of patients will include measurement of blood levels of IDS enzyme, assessment of metabolic correction by urinary glycosaminoglycan levels, clinical response of the disease, and monitoring for potential toxicity. This Phase I study is to demonstrate the safety of the L2SN-mediated gene therapy and to provide a preliminary evaluation of clinical efficacy. (Protocol #9409-087)

**Appendix D-105.** Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California, may conduct gene transfer experiments on 25 subjects ( $\geq 18$  years of age) with metastatic melanoma. The protocol is an open label, Phase I trial to evaluate the safety and immunological effects of administering lethally irradiated allogeneic and autologous melanoma cells transduced with the retroviral vector, IL-7/HyTK, which encodes the gene for human interleukin-7 (IL-7). Subjects will receive  $1 \times 10^7$  irradiated unmodified autologous tumor cells in combination with escalating doses of IL-7/HyTK transduced allogeneic melanoma cells (M24 cell line). The number of M24 cells administered will be adjusted based on the level of IL-7 expression. Subjects will receive 3 biweekly subcutaneous injections of M24 cells expressing 10, 100, or 1000 nanograms of IL-7/hour *in vivo*. A final cohort of 5 subjects will receive IL-7/HyTK transduced autologous cells. Subjects will be monitored for antitumor activity by skin tests, biopsy analysis, tumor-specific antibody activity, and cytotoxic T lymphocyte precursor evaluation. Non-immunologic parameters will also be monitored. (Protocol #9503-101)

**Appendix D-106.** Dr. Jack A. Roth, MD Anderson Cancer Center, may conduct gene transfer experiments on 42 subjects ( $\geq 18$  years of age) with refractory non-small cell lung cancer (NSCLC). Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human p53 tumor suppressor gene. The E1 region of AD5CMV-p53 has been replaced with a p53 expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) 21 subjects will receive Ad5CMV-p53 alone, and (2) 21 subjects will receive Ad5CMV-p53 in combination with cisplatin. Following vector administration, subjects will be isolated for 96 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of this study are determine: (1) the maximum tolerated dose of AD5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutants using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer  $> 5 \times 10^{10}$ ) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc (control adenovirus vector containing the luciferase gene) to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9406-079)

**Appendix D-107A.** Dr. Gary Clayman, M.D. Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 21 subjects ( $\geq 18$  years of age) with refractory squamous cell carcinoma of the head and neck. Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human p53 tumor suppressor gene. The E1 region of AD5CMV-p53 has been replaced with a p53 expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) those with non-resectable tumors, and (2) those with surgically accessible tumors. Subjects will receive multiple injections of vector in each dose-escalation cohort. Following vector administration, subjects will be isolated for 48 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of the study are to determine: (1) the maximum tolerated dose of AD5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutants using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer  $> 5 \times 10^{10}$ ) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9412-096)

**Appendix D-107B.** Drs. Bernard A. Fox and Walter J. Urba of Earle A. Chiles Research Institute, Providence Medical Center, Portland, Oregon, may conduct gene transfer experiments on 18 subjects ( $\geq 18$  years of age) with metastatic renal cell carcinoma or melanoma. Autologous tumor cells will be surgically removed, transduced *in vitro* with the cationic liposome plasmid vector, VCL-1005, which encodes human leukocyte antigen (HLA)-B7 and beta-2 microglobulin. Subjects will receive subcutaneous injection of lethally irradiated transduced cells in one limb. The contralateral limb will be injected with lethally irradiated untransduced tumor cells in combination with Bacille Calmette-Guerin (BCG). Approximately 21 days following tumor cell injection, subjects will undergo lymphadenectomy for subsequent *in vitro* expansion of anti-CD3 activated lymphocytes. Activated lymphocytes will be adoptively transferred on approximately day 35 in combination with a 5-day course of interleukin-2 (IL-2). On approximately day 45, subjects will receive a second cycle of IL-2. The objectives of this study are to determine: (1) the safety of administering anti-CD3 activated antitumor effector T cells in draining lymph nodes, and (2) whether HLA-B7/ $\beta$ -2 gene transfer augments the sensitization of anti-tumor effector T-cells in draining lymph nodes. (Protocol 9506-108)

**Appendix D-108.** Dr. Mitchell S. Steiner, University of Tennessee, Memphis, Tennessee, and Dr. Jeffrey T. Holt, Vanderbilt University School of Medicine, Nashville, Tennessee, may conduct gene transfer experiments on 15 male subjects (35 to 75 years of age) with metastatic prostate cancer. Malignant cells obtained from advanced prostate cancer subjects have been demonstrated to express high levels of the protooncogene c-myc *in vivo*. The mouse mammary tumor virus (MMTV) long terminal repeat (LTR) is expressed at high levels in prostate tissue. Following removal of malignant cells via biopsy, subjects will receive a single transrectal ultrasound-guided intraprostate quadrant injection of the retrovirus vector, XM6:MMTV-antisense c-myc, for 4 consecutive days at the site of the original biopsy. The objectives of this Phase I study are to: (1) quantitatively assess the uptake and expression of XM6:MMTV-antisense c-myc by prostate cancer cells *in vivo*, (2) determine whether c-myc gene expression is prostate tumor-specific, (3) assess safety of intraprostate injection of XM6:MMTV-antisense c-myc, and (4) biologic efficacy (antisense inhibition of tumor growth). (Protocol #9509-123)

**Appendix D-109.** Drs. Ronald G. Crystal, Edward Hershowitz, and Michael Lieberman, New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 18 subjects (18 to 70 years of age) with metastatic colon carcinoma with liver metastases. In this Phase I dose-escalation study, subjects will receive computed tomography (CT)-guided intratumoral injections of the adenovirus vector, Ad<sub>G<sub>V</sub></sub>CD.10, into the same hepatic metastasis in 4 equal volumes (100 microliters), each with a separate entry into the liver. This dosage schedule will be performed on Days 1 and 7. 5-fluorocytosine (200 milligrams/kilogram/24 hours) will be administered orally in 4 equal doses starting on day 2 and continuing through the time of laparotomy. The objectives of this study are to: (1) determine the dose-dependent toxicity of direct administration of Ad<sub>G<sub>V</sub></sub>CD.10 to hepatic metastases combined with oral administration of 5-fluorocytosine, (2) quantitatively assess transfer and expression of the cytosine deaminase gene in target cells, and (3) determine the biologic effects of direct Ad<sub>G<sub>V</sub></sub>CD.10 administration to hepatic metastases. (Protocol #9509-125)

**Appendix D-110.** Drs. Andres Berchuck and H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 18 subjects ( $\geq 18$  years of age) with refractory metastatic ovarian cancer. Autologous tumor cells obtained from ascites or surgically removed tumor will be transduced with the cationic liposome vector, PMP6A-IL2, that contains an adeno-associated virus derived plasmid DNA, a cytomegalovirus (CMV) promoter, and interleukin-2 (IL-2) complementary DNA (cDNA). In this dose-escalation study, subjects will undergo 4 cycles of intradermal injections (thigh or abdomen) of *ex vivo* transduced, lethally irradiated tumor cells in an attempt to induce an antitumor response. The objectives of the study are to evaluate: (1) the safety of intradermally injected transduced cells, and (2) antitumor response following therapy. (Protocol #9506-110)

**Appendix D-111.** Drs. Stephen L. Eck and Jane B. Alavi of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 18 subjects (>18 years of age) with malignant glioma. The adenovirus vector encoding the *Herpes simplex* virus thymidine kinase (HSV-TK) gene, H5.020RSVTK, will be injected by a stereotactic guided technique into brain tumors. Afterwards, the patients will receive systemic ganciclovir (GCV) treatment. Patients eligible to undergo a palliative debulking procedure will receive the same treatment followed by resection on day 7, and a second dose of the vector intra-operatively. Brain tissues removed by resection will be analyzed for adenovirus infection, transgene expression, and signs of inflammation. The size and metabolic activity of tumors will be monitored by scanning with magnetic resonance imaging and positron emission tomography. The objective of the study is to evaluate the overall safety of this treatment and to gain insight into the parameters that may limit the general applicability of this approach. (Protocol #9409-089)

**Appendix D-112.** Drs. Robert Grossman and Savio Woo of the Baylor College of Medicine & Methodist Hospital, Houston, Texas, may conduct gene transfer experiments on 20 subjects ( $\geq 18$  years of age) with refractive central nervous system malignancies. Subjects will receive stereotaxic injections of a replication-defective, type 5 E1/E3-deleted adenovirus vector, ADV/RSV-tk, to deliver the *Herpes simplex* virus thymidine kinase (HSV-TK) gene to tumor cells. Expression of the HSV-TK gene is driven by a Rous sarcoma virus long terminal repeat (RSV-LTR). Subjects will receive a single time-course of intravenous ganciclovir (GCV) (14 consecutive days) following vector administration. Following demonstration of safety with the initial starting dose of  $1 \times 10^8$  particles in 5 subjects, additional cohorts will receive between  $5 \times 10^8$  and  $1.5 \times 10^9$  particles. Each cohort will be monitored for toxicity for one month before administration of the next higher dose to subsequent cohorts. Subjects will be monitored for evidence of clinical efficacy by magnetic resonance imaging and/or computer tomography scans. The primary objective of this Phase I study is to determine the safety of vector administration. (Protocol #9412-098)

**Appendix D-113.** Drs. Gabriel N. Hortobagyi, Gabriel Lopez-Berestein, and Mien-Chie Hung, of the University of Texas MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on a maximum of 24 adult patients (12 for each cancer) with metastatic breast or ovarian carcinoma. Overexpression of the *HER-2/neu* oncogene occurs in 30% of ovarian and breast cancers, and it is associated with enhanced metastatic potential, drug resistance, and poor survival. The E1A gene of the adenovirus type 5 functions as a tumor suppressor gene when transfected into cancer cells which overexpress the *HER-2/neu* oncogene. E1A expression induces down regulation of the level of the *HER-2/neu* oncoprotein by a transcriptional control mechanism. A plasmid, pE1A, encoding the adenovirus E1A gene with its own promoter will be administered as a DNA/lipid complex via the intraperitoneal or intrapleural route. The objectives of the study are: (1) to determine E1A gene transduction into malignant cells after the administration of E1A/lipid complex by intrapleural or intraperitoneal administration, (2) to determine whether E1A gene therapy can down-regulate *HER-2/neu* expression after intrapleural or intraperitoneal administration, (3) to determine the maximum biologically active dose (MBAD), or the maximum tolerated dose (MTD) of E1A/lipid complex, (4) to determine the toxicity and tolerance of E1A/lipid complex administered into the pleural or peritoneal space, and to assess the reversibility of such toxicity, and (5) to evaluate tumor response. (Protocol #9512-137)

**Appendix D-114.** Drs. Keith L. Black and Habib Fakhrai of the University of California, Los Angeles, California, may conduct gene transfer experiments on 12 subjects ( $\geq 18$  years of age) with glioblastoma multiforme. An Epstein-Barr virus (EBV) based plasmid vector, pCEP-4/TGF- $\beta$ 2 antisense, encoding antisense RNA will be used to inhibit TGF- $\beta$ 2 production. Tumor samples obtained from the patients at the time of clinically indicated surgery will be grown in culture to establish a cell line for each patient. The patients' tumor cells will be genetically altered with the pCEP-4/TGF- $\beta$ 2 vector to inhibit their secretion of TGF- $\beta$ . Following completion of the traditional post surgical radiation therapy, the first cohort of patients will receive, at 3 week intervals, 4 injections of  $5 \times 10^6$  irradiated gene modified autologous tumor cells. Subsequently, in dose escalation studies, the second cohort will receive  $1 \times 10^7$  cells, and the third cohort,  $2 \times 10^7$  cells. The results of this Phase I trial will be used to assess the safety of this form of gene therapy and may provide preliminary data to evaluate the potential utility of TGF- $\beta$ 2 antisense gene therapy in the management of gliomas. (Protocol #9512-138)

**Appendix D-115.** Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on a total of 21 (with an option for an additional 5) normal males and female subjects, age  $\geq 18$  years. Replication-deficient adenovirus (Ad) vector previously has been used in a number of human gene therapy strategies to transfer genes *in vivo* for therapeutic purposes. The purpose of this protocol is to characterize the local (skin), systemic (blood), and distant compartment (lung) immunity in normal individuals after intradermal administration of a replication deficient Ad5-based vector, named

Ad<sub>GV</sub>CD.10, carrying the gene coding for the *E. coli* enzyme, cytosine deaminase (CD). Following intradermal administration of the vector to normal individuals, the skin, blood, and lung immune responses to the Ad vector and CD transgene will be evaluated over time. This vector has been safety administered intrahepatically ten times to five individuals with colon carcinoma. No adverse effects in Protocol #9509-125 have been observed. The present protocol will yield insights into normal human immune responses to both the Ad vector, as well as to a heterologous (i.e., non-human) gene product (CD). Note: This study is designed to answer basic biological questions regarding characterization of the immune responses to such vectors that have been previously documented. (Protocol #9701-171)

**Appendix D-116.** Dr. Daniel Rockey at Oregon State University and Dr. Walter Stamm at the University of Washington may conduct experiments to deliberately transfer a gene encoding tetracycline resistance from *Chlamydia suis* (a swine pathogen) into *C. trachomatis* (a human pathogen). This approval is specific to Drs. Rockey and Stamm and research with these resistant organisms may only occur under the conditions as specified by the NIH Director (72 FR 61661). This approval was effective as of September 24, 2007.

**Appendix D-117.** Dr. David Walker at the University of Texas Medical Branch may conduct experiments to deliberately introduce a gene encoding chloramphenicol resistance into *Rickettsia conorii*. This approval is specific to Dr. Walker and research with these resistant organisms may only occur under the conditions as specified by the NIH Director (73 FR 32719). This approval was effective as of April 7, 2008.

**Appendix D-118.** Dr. Harlan Caldwell at the Rocky Mountain Laboratories may conduct experiments to deliberately introduce a gene encoding tetracycline resistance into *Chlamydia trachomatis* serovar L2. This approval is specific to Dr. Caldwell and research with this resistant organism may only occur under the conditions as specified by the NIH Director (76 FR 27653). This approval was effective as of April 26, 2010.

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## **APPENDIX E. CERTIFIED HOST-VECTOR SYSTEMS** (See [Appendix I](#), Biological Containment)

While many experiments using *Escherichia coli* K-12, *Saccharomyces cerevisiae*, and *Bacillus subtilis* are currently exempt from the *NIH Guidelines* under [Section III-F, Exempt Experiments](#), some derivatives of these host-vector systems were previously classified as Host-Vector 1 Systems or Host-Vector 2 Systems. A listing of those systems follows:

### **Appendix E-I. *Bacillus subtilis***

#### **Appendix E-I-A. *Bacillus subtilis* Host-Vector 1 Systems**

The following plasmids are accepted as the vector components of certified *B. subtilis* systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of Host-Vector 1 systems based on these plasmids.

#### **Appendix E-I-B. *Bacillus subtilis* Host-Vector 2 Systems**

The asporogenic mutant derivative of *Bacillus subtilis*, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

### **Appendix E-II. *Saccharomyces cerevisiae***

#### **Appendix E-II-A. *Saccharomyces cerevisiae* Host-Vector 2 Systems**

The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

**Appendix E-III. *Escherichia coli*****Appendix E-III-A. *Escherichia coli* (EK2) Plasmid Systems**

The *Escherichia coli* K-12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, and pHB1. The following *Escherichia coli*/S. cerevisiae hybrid plasmids are certified as EK2 vectors when used in *Escherichia coli* chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3, and SHY4: Ylp1, YEp2, YEp4, Ylp5, YEp6, YRp7, YEp20, YEp21, YEP24, Ylp25, Ylp26, Ylp27, Ylp28, Ylp29, Ylp30, Ylp31, Ylp32, and Ylp33.

**Appendix E-III-B. *Escherichia coli* (EK2) Bacteriophage Systems**

The following are certified EK2 systems based on bacteriophage lambda:

<b>Vector</b>	<b>Host</b>
λgt WESλB'	DP50 <sup>supF</sup>
λgt WESλB*	DP50 <sup>supF</sup>
λgt ZJ virλB'	<i>Escherichia coli</i> K-12
λgtALO·λB	DP50 <sup>supF</sup>
Charon 3A	DP50 or DP50 <sup>supF</sup>
Charon 4A	DP50 or DP50 <sup>supF</sup>
Charon 16A	DP50 or DP50 <sup>supF</sup>
Charon 21A	DP50 <sup>supF</sup>
Charon 23A	DP50 or DP50 <sup>supF</sup>
Charon 24A	DP50 or DP50 <sup>supF</sup>

*Escherichia coli* K-12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50<sup>supF</sup> provided that the *su*-strain not be used as a propagation host.

**Appendix E-IV. *Neurospora crassa*****Appendix E-IV-A. *Neurospora crassa* Host-Vector 1 Systems**

The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

In1 (inositol-less) strains 37102, 37401, 46316, 64001, and 89601. Csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

**Appendix E-V. *Streptomyces*****Appendix E-V-A. *Streptomyces* Host-Vector 1 Systems**

The following *Streptomyces* species: *Streptomyces coelicolor*, *S. lividans*, *S. parvulus*, and *S. griseus*. The following are accepted as vector components of certified *Streptomyces* Host-Vector 1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

**Appendix E-VI. *Pseudomonas putida*****Appendix E-VI-A. *Pseudomonas putida* Host-Vector 1 Systems**

*Pseudomonas putida* strains KT2440 with plasmid vectors pKT262, pKT263, and pKT264.

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## APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

### Appendix F-I. General Information

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD<sub>50</sub> of < 100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under [Section III-B-1 \(Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms Per Kilogram Body Weight\)](#) and require Institutional Biosafety Committee and NIH/OBA approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD<sub>50</sub> ≥ 100 micrograms per kilograms of body weight. Experiments involving genes coding for toxin molecules with an LD<sub>50</sub> of < 100 micrograms per kilograms and > 100 nanograms per kilograms body weight require Institutional Biosafety Committee approval and registration with NIH/OBA prior to initiating the experiments. A list of toxin molecules classified as to LD<sub>50</sub> is available from NIH/OBA. Testing procedures for determining toxicity of toxin molecules not on the list are available 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). The results of such tests shall be forwarded to NIH/OBA, which will consult with *ad hoc* experts, prior to inclusion of the molecules on the list (see [Section IV-C-1-b-\(2\)-\(c\), Minor Actions](#)).

### Appendix F-II. Cloning of Toxin Molecule Genes in *Escherichia coli* K-12

**Appendix F-II-A.** Cloning of genes coding for molecules toxic for vertebrates that have an LD<sub>50</sub> of >100 nanograms per kilograms and <1000 nanograms per kilograms body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under Biosafety Level (BL) 2 + EK2 or BL3 + EK1 containment conditions.

**Appendix F-II-B.** Cloning of genes for the biosynthesis of molecules toxic for vertebrates that have an LD<sub>50</sub> of >1 microgram per kilogram and <100 microgram per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordetella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

**Appendix F-II-C.** Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *Escherichia coli*, *Klebsiella*, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *Escherichia coli* and of *Yersinia enterocolitica*.

### Appendix F-III. Cloning of Toxic Molecule Genes in Organisms Other Than *Escherichia coli* K-12

Requests involving the cloning of genes coding for toxin molecules for vertebrates at an LD<sub>50</sub> of <100 nanograms per kilogram body weight in host-vector systems other than *Escherichia coli* K-12 will be evaluated by NIH/OBA in consultation with *ad hoc* toxin experts (see [Sections III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms Per Kilogram Body Weight](#), and [IV-C-1-b-\(2\)-\(c\), Minor Actions](#)).

### Appendix F-IV. Specific Approvals

An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD<sub>50</sub> of <100 nanograms per kilogram body weight is available 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).

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## APPENDIX G. PHYSICAL CONTAINMENT

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large-scale (over 10 liters) research or production, Appendix K (*Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*) supersedes Appendix G. [Appendix K](#) defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P (*Physical and Biological Containment for Recombinant DNA Research Involving Plants*) supersedes Appendix G. [Appendix P](#) defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q (*Physical and Biological Containment for Recombinant DNA Research Involving Animals*) supersedes Appendix G. [Appendix Q](#) defines Biosafety Levels 1 through 4 - Animals.

### Appendix G-I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see [Appendices G-III-A](#) through [G-III-J](#), *Footnotes and References of Appendix G*). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see [Sections IV-B-1-h](#), *Responsibilities of the Institution--General Information*, and [IV-B-7-d](#), *Responsibilities of the Principal Investigator Prior to Initiating Research*). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see [Sections IV-B-7-d](#), *Responsibilities of the Principal Investigator Prior to Initiating Research* and [IV-B-7-e](#), *Responsibilities of the Principal Investigator During the Conduct of the Research*). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see [Section IV-B-1-f](#), *Responsibilities of the Institution--General Information*).

The *Laboratory Safety Monograph* (see [Appendix G-III-O](#), *Footnotes and References of Appendix G*) and *Biosafety in Microbiological and Biomedical Laboratories* (see [Appendix G-III-A](#), *Footnotes and References of Appendix G*) describe practices, equipment, and facilities in detail.

### Appendix G-II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see [Appendix G-III-A](#), *Footnotes and References of Appendix G*). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see [Appendix G-III-C](#), *Footnotes and References of Appendix G*).



It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The *NIH Guidelines*, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given to other combinations which achieve an equivalent level of containment (see Sections [IV-C-1-b-\(1\)](#), *Major Actions* and [IV-C-1-b-\(2\)](#), *Minor Actions*).

#### **Appendix G-II-A. Biosafety Level 1 (BL1)** (See [Appendix G-III-M](#), *Footnotes and References of Appendix G*)

##### **Appendix G-II-A-1. Standard Microbiological Practices (BL1)**

**Appendix G-II-A-1-a.** Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

**Appendix G-II-A-1-b.** Work surfaces are decontaminated once a day and after any spill of viable material.

**Appendix G-II-A-1-c.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-A-1-d.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-A-1-e.** Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

**Appendix G-II-A-1-f.** Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.

**Appendix G-II-A-1-g.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-A-1-h.** In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

##### **Appendix G-II-A-2. Special Practices (BL1)**

**Appendix G-II-A-2-a.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-A-2-b.** An insect and rodent control program is in effect.

##### **Appendix G-II-A-3. Containment Equipment (BL1)**

**Appendix G-II-A-3-a.** Special containment equipment is generally not required for manipulations of agents assigned to BL1.

##### **Appendix G-II-A-4. Laboratory Facilities (BL1)**

**Appendix G-II-A-4-a.** The laboratory is designed so that it can be easily cleaned.

**Appendix G-II-A-4-b.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-A-4-c.** Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-A-4-d.** Each laboratory contains a sink for hand washing.

**Appendix G-II-A-4-e.** If the laboratory has windows that open, they are fitted with fly screens.



**Appendix G-II-B. Biosafety Level 2 (BL2)** (See [Appendix G-III-N](#), *Footnotes and References of Appendix G*)**Appendix G-II-B-1. Standard Microbiological Practices (BL2)**

**Appendix G-II-B-1-a.** Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

**Appendix G-II-B-1-b.** Work surfaces are decontaminated at least once a day and after any spill of viable material.

**Appendix G-II-B-1-c.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-B-1-d.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-B-1-e.** Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

**Appendix G-II-B-1-f.** Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

**Appendix G-II-B-1-g.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-B-1-h.** Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

**Appendix G-II-B-2. Special Practices (BL2)**

**Appendix G-II-B-2-a.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-B-2-b.** The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

**Appendix G-II-B-2-c.** The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

**Appendix G-II-B-2-d.** When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

**Appendix G-II-B-2-e.** An insect and rodent control program is in effect.

**Appendix G-II-B-2-f.** Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

**Appendix G-II-B-2-g.** Animals not involved in the work being performed are not permitted in the laboratory.

**Appendix G-II-B-2-h.** Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

**Appendix G-II-B-2-i.** All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

**Appendix G-II-B-2-j.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

**Appendix G-II-B-2-k.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to NIH/OBA shall be sent 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). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix G-II-B-2-l.** When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

**Appendix G-II-B-2-m.** A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

### **Appendix G-II-B-3. Containment Equipment (BL2)**

**Appendix G-II-B-3-a.** Biological safety cabinets (Class I or II) (see [Appendix G-III-L](#), *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

**Appendix G-II-B-3-a-(1).** Procedures with a high potential for creating aerosols are conducted (see [Appendix G-III-O](#), *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

**Appendix G-II-B-3-a-(2).** High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

### **Appendix G-II-B-4. Laboratory Facilities (BL2)**

**Appendix G-II-B-4-a.** The laboratory is designed so that it can be easily cleaned.

**Appendix G-II-B-4-b.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-B-4-c.** Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-B-4-d.** Each laboratory contains a sink for hand washing.

**Appendix G-II-B-4-e.** If the laboratory has windows that open, they are fitted with fly screens.

**Appendix G-II-B-4-f.** An autoclave for decontaminating laboratory wastes is available.

**Appendix G-II-C. Biosafety Level 3 (BL3)** (See [Appendix G-III-P](#), *Footnotes and References of Appendix G*)

**Appendix G-II-C-1. Standard Microbiological Practices (BL3)**

**Appendix G-II-C-1-a.** Work surfaces are decontaminated at least once a day and after any spill of viable material.

**Appendix G-II-C-1-b.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-C-1-c.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-C-1-d.** Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

**Appendix G-II-C-1-e.** Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

**Appendix G-II-C-1-f.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-C-1-g.** Persons under 16 years of age shall not enter the laboratory.

**Appendix G-II-C-1-h.** If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

**Appendix G-II-C-2. Special Practices (BL3)**

**Appendix G-II-C-2-a.** Laboratory doors are kept closed when experiments are in progress.

**Appendix G-II-C-2-b.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-C-2-c.** The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

**Appendix G-II-C-2-d.** The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

**Appendix G-II-C-2-e.** When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

**Appendix G-II-C-2-f.** All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

**Appendix G-II-C-2-g.** The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

**Appendix G-II-C-2-h.** An insect and rodent program is in effect.

**Appendix G-II-C-2-i.** Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

**Appendix G-II-C-2-j.** Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

**Appendix G-II-C-2-k.** Molded surgical masks or respirators are worn in rooms containing experimental animals.

**Appendix G-II-C-2-l.** Animals and plants not related to the work being conducted are not permitted in the laboratory.

**Appendix G-II-C-2-m.** Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see [Appendix G-III-K, Footnotes and References of Appendix G](#)), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

**Note:** Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

**Appendix G-II-C-2-n.** All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

**Appendix G-II-C-2-o.** Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix G-II-C-2-p.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix G-II-C-2-q.** Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to NIH/OBA shall be sent 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). Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

**Appendix G-II-C-2-r.** Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

**Appendix G-II-C-2-s.** A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

#### **Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)**

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in [Appendix G-Table 1, Possible Alternate Combinations of Physical and Biological Containment Safeguards](#).

### **Appendix G-II-C-3. Containment Equipment (BL3)**

**Appendix G-II-C-3-a.** Biological safety cabinets (Class I, II, or III) (see [Appendix G-III-L, Footnotes and References of Appendix G](#)) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

### **Appendix G-II-C-4. Laboratory Facilities (BL3)**

**Appendix G-II-C-4-a.** The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

**Appendix G-II-C-4-b.** The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

**Appendix G-II-C-4-c.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-C-4-d.** Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-C-4-e.** Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

**Appendix G-II-C-4-f.** Windows in the laboratory are closed and sealed.

**Appendix G-II-C-4-g.** Access doors to the laboratory or containment module are self-closing.

**Appendix G-II-C-4-h.** An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

**Appendix G-II-C-4-i.** A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

**Appendix G-II-C-4-j.** The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see [Appendix G-III-L, Footnotes and References of Appendix G](#))) that avoids any interference with the air balance of the cabinets or building exhaust system.

**Appendix G-II-C-5. Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses.****Appendix G-II-C-5.a. Containment, Practices, and Training for Research with Risk Group 3 Influenza Viruses (BL3 Enhanced).**

**Appendix G-II-C-5-a-(1).** In addition to standard BL3 practices, the following additional personal protective equipment and practices shall be used: (1) Powered Air-purifying Respirators (PAPR) are worn. (2) Street clothes are changed to protective suit (e.g., wrap-back disposable gown, olefin protective suit). (3) Double gloves are worn. (4) Appropriate shoe coverings are worn (e.g., double disposable shoe coverings, single disposable shoe coverings if worn with footwear dedicated to BL3 enhanced laboratory use, or impervious boots or shoes of rubber or other suitable material that can be decontaminated). (5) Showers prior to exiting the laboratory should be considered depending on risk assessment of research activities.

**Appendix G-II-C-5-a-(2).** As proper training of laboratory workers is an essential component of biosafety, retraining and periodic reassessments (at least annually) in BL3 enhanced practices, especially the proper use of respiratory equipment, such as PAPRs, and clothing changes is required.

**Appendix G-II-C-5-a-(3).** Reporting of all spills and accidents, even if relatively minor, is required as described in Appendix G-II-C-2-q.

**Appendix G-II-C-5-a-(4).** To avoid inadvertent cross contamination of 1918 H1N1, HPAI H5N1 or human H2N2 (1957-1968): (1) Containment facilities and practices appropriate for highest risk group virus shall be used at all times with lower risk group viruses, when studied in the same laboratory room. (2) Tissue cultures with these viruses shall be conducted at separate times (temporal spacing) in the same room. (3) Separate reagents shall be used to minimize risk of cross contamination. (4) A laboratory worker shall not perform concurrent influenza virus experiments that carry the risk of unintended reassortment among 1918 H1N1, human H2N2 (1957-1968), HPAI H5N1 and other human influenza viruses. (5) Two or more laboratory workers shall not perform within the same work area simultaneous influenza virus experiments that carry the risk of unintended segment reassortment between 1918 H1N1, or HPAI H5N1, or human H2N2 (1957-1968) and other human influenza viruses. (6) Between experiments good biosafety decontamination practices (e.g., surface and biosafety cabinet surface decontamination according to standard BL3 procedures) shall be used and there shall be a thirty minute wait period after decontamination before equipment is used for experiments with any other influenza A viruses. (7) Between experiments, in addition to decontamination of the work area, clothing changes and PAPR disinfection shall be performed prior to handling a different influenza virus in the same work area. (Shower-out capability may be required by [USDA/APHIS](#) for certain experiments with HPAI H5N1.)

**Appendix G-II-C-5-a-(5).** Continued susceptibility of the reassortant influenza viruses containing genes and/or segments from 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968) to antiviral agents shall be established by sequence analysis or suitable biological assays. After manipulation of genes that influence sensitivity to antiviral agents, susceptibility to these agents shall be reconfirmed.

**Appendix G-II-C-5.b. Containment for Animal Research.**

Guidance provided in [Appendix G-II-C](#) and [Appendix Q-II-C](#) is applicable with the following emphasis on standard BL3 or BL3-N containment or additional enhancements.

**Appendix G-II-C-5-b-(1).** Research with small animals shall be conducted in a class II biosafety cabinet. Small animals such as rodents (e.g. mice, hamsters, rats, guinea pigs) can be housed within a negative pressure BL3 animal suite using high-density individually vented caging (IVC) systems that independently supply high efficiency particulate air/HEPA-filtered and directional air circulation. Other animals (e.g. rabbits, ferrets) that are of a size or have growth or caging requirements that preclude the use of high-density IVC systems are to be housed in negative pressure bioisolators.

**Appendix G-II-C-5-b-(2).** Large animals such as non-human primates shall be housed in primary barrier environments according to BL3-N containment requirements (see [Appendix Q-II-c](#)).

**Appendix G-II-C-5-b-(3).** Specialized training and proven competency in all assigned practices and procedures shall be required for laboratory staff, including staff involved in animal care.



**Appendix G-II-C-5-b-(4).** For HPAI H5N1 research, the *NIH Guidelines* defer to [USDA/APHIS](#) recommendations for biocontainment practices for loose housed animals.

**Appendix G-II-C-5-c. Occupational Health**

A detailed occupational health plan shall be developed in advance of working with these agents in consultation, as needed, with individuals with the appropriate clinical expertise. In addition, the appropriate public health authority shall be consulted (e.g. local public health officials) on the plan and a mock drill of this plan shall be undertaken periodically. The plan should include an incident reporting system and laboratory workers shall report all incidents.

**Appendix G-II-C-5-c-(1).** Laboratory workers shall be provided with medical cards which include, at a minimum, the following information: characterization of the influenza virus to which they have been potentially exposed, and 24-hour contact numbers for the principal investigator and institution's occupational health care provider(s).

**Appendix G-II-C-5-c-(2).** A detailed occupational health plan shall include: (1) Unless there is a medical contraindication to vaccination (e.g. severe egg allergy) annual seasonal influenza vaccination as prerequisite for research to reduce risk of influenza like illness requiring isolation and tests to rule out infection with experimental virus and possible co-infection with circulating influenza strains. (2) Virus specific vaccination, if available, should be offered. (3) Reporting of all respiratory symptoms and/or fever (i.e. influenza-like illnesses). (4) 24-hour access to a medical facility that is prepared to implement appropriate respiratory isolation to prevent transmission and is able to provide appropriate antiviral agents. Real-time reverse transcription-polymerase chain reaction (RT-PCR) procedures should be used to discriminate these viruses from currently circulating human influenza viruses. For exposures to viruses containing genes from 1918 H1N1 or the HA gene from human H2N2 (1957-1968), specimens shall be sent to the [CDC](#) for testing (RT-PCR and confirmatory sequencing).

**Appendix G-II-C-5-c-(3).** In preparing to perform research with 1918 H1N1, human H2N2 (1957-1968), or HPAI H5N1, principal investigators should develop a clear plan specifying who will be contacted in the event of a potential exposure (during and after work hours) to conduct a risk assessment and make decisions as to the required response, including the need for and extent of isolation of the exposed worker. After any kind of potential exposure, a rapid risk assessment shall be performed by the principal investigator, health and biosafety officials and subsequent actions should depend on the appraised level of risk of respiratory infection for the individual and potential for transmission to others. A laboratory worker performing research with either an influenza virus containing the HA gene from human H2N2 or an influenza virus containing genes and/or segments from 1918 H1N1, shall be informed in advance that, in the case of a *known* laboratory exposure with a high risk for infection, e.g., involving the upper or lower respiratory tract or mucous membranes, the laboratory worker will need to be isolated in a predetermined facility, rather than home isolation, until infection can be ruled out by testing (e.g., negative RT-PCR for 1918 H1N1 or human H2N2 (1957-1968)) of appropriately timed specimens. Laboratory workers shall be informed in advance that in the case of a known laboratory exposure to highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage with high risk for infection, they should be prepared to self isolate (for example at home) until infection can be ruled out by testing (e.g., negative RT-PCR for HPAI H5N1) of appropriately timed specimens. The action taken for other types of exposures should be based on the risk assessment. In addition, based on the risk assessment: (1) treatment with appropriate antiviral agents shall be initiated, and (2) the appropriate public health authorities shall be notified.

**Appendix G-II-C-5-c-(4). Influenza-like illness.** If a laboratory worker, who had recent exposure (within ten days) to influenza viruses containing the human H2N2 HA gene or any gene from the 1918 H1N1 or HPAI H5N1 viruses, or to animals exposed to such viruses, demonstrates symptoms and/or signs of influenza infection (e.g., fever/chills, cough, myalgias, headache), then the lab worker shall report by phone to the supervisor/principal investigator and other individuals identified in the occupational health plan. The laboratory worker shall be transported to a healthcare facility that can provide adequate respiratory isolation, appropriate medical therapy, and testing to determine whether the infection is due to a recombinant influenza virus. The appropriate public health authorities shall be informed whenever a suspected case is isolated.

**Appendix G-II-C-5-c-(5).** For 1918 H1N1 research, the use of antiviral agents (e.g., oseltamivir) for pre-exposure prophylaxis shall be discussed with laboratory workers in advance including a discussion of the data on the safety of long term exposure to these agents and their ability to reduce the risk of clinical disease and the limits of the data regarding protection of close contacts and the community.

**Appendix G-II-C-5-c-(6).** Antiviral agents for post-exposure prophylaxis shall be provided only after medical evaluation. Home supplies shall not be provided in advance for research with 1918 H1N1 or influenza viruses containing the HA gene from human H2N2.

#### **Appendix G-II-D. Biosafety Level 4 (BL4)**

##### **Appendix G-II-D-1. Standard Microbiological Practices (BL4)**

**Appendix G-II-D-1-a.** Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

**Appendix G-II-D-1-b.** Only mechanical pipetting devices are used.

**Appendix G-II-D-1-c.** Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

**Appendix G-II-D-1-d.** All procedures are performed carefully to minimize the creation of aerosols.

##### **Appendix G-II-D-2. Special Practices (BL4)**

**Appendix G-II-D-2-a.** Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

**Appendix G-II-D-2-b.** No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before exiting the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix G-II-D-2-c.** Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the Principal Investigator, Biological Safety Officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

**Appendix G-II-D-2-d.** Personnel enter and exit the facility only through the clothing change and shower rooms. Personnel shower each time they exit the facility. Personnel use the air locks to enter or exit the laboratory only in an emergency.

**Appendix G-II-D-2-e.** Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing (may be disposable), including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When exiting the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. Protective clothing shall be decontaminated prior to laundering or disposal.



**Appendix G-II-D-2-f.** When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biosafety symbol is posted on all access doors. The sign identifies the agent, lists the name of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

**Appendix G-II-D-2-g.** Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

**Appendix G-II-D-2-h.** An insect and rodent control program is in effect.

**Appendix G-II-D-2-i.** Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

**Appendix G-II-D-2-j.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

**Appendix G-II-D-2-k.** A system is set up for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to the NIH/OBA shall be sent 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). Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

**Appendix G-II-D-2-l.** Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems, such as Horsfall units (see [Appendix G-III-K](#), *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

#### **Appendix G-II-D-2-m. Alternative Selection of Containment Equipment (BL4)**

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

#### **Appendix G-II-D-3. Containment Equipment (BL4)**

**Appendix G-II-D-3-a.** All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

**Appendix G-II-D-4. Laboratory Facilities (BL4)**

**Appendix G-II-D-4-a.** The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

**Appendix G-II-D-4-b.** Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain high efficiency particulate air/HEPA filters.

**Appendix G-II-D-4-c.** Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

**Appendix G-II-D-4-d.** Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-D-4-e.** Laboratory furniture is simple and of sturdy construction; and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-D-4-f.** A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

**Appendix G-II-D-4-g.** If there is a central vacuum system, it does not serve areas outside the facility. In-line high efficiency particulate air/HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent back-flow.

**Appendix G-II-D-4-h.** If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the back-flow protected distribution system supplying water to the laboratory areas.

**Appendix G-II-D-4-i.** Access doors to the laboratory are self-closing and locking.

**Appendix G-II-D-4-j.** Any windows are breakage resistant.

**Appendix G-II-D-4-k.** A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

**Appendix G-II-D-4-l.** A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

**Appendix G-II-D-4-m.** Liquid effluent from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

**Appendix G-II-D-4-n.** An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

**Appendix G-II-D-4-o.** The exhaust air from the facility is filtered through high efficiency particulate air/HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

**Appendix G-II-D-4-p.** The treated exhaust air from Class I and II biological safety cabinets may be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at six-month intervals. *The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of high efficiency particulate air/HEPA filters in series, via the facility exhaust air system.* If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see [Appendix G-III-L, Footnotes and References of Appendix G](#))) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

**Appendix G-II-D-4-q.** A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area. The exhaust air from the suit area is filtered by two sets of high efficiency particulate air/HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is greater than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit areas.

**Appendix G - Table 1. Possible Alternate Combinations Of Physical And Biological Containment Safeguards**

Classification of Physical & Biological Containment	Alternate Physical Containment			Alternate Biological Containment
	Laboratory Facilities	Laboratory Practices	Laboratory Equipment	
BL3/HV2	BL3 BL3	BL3 BL3	BL3 BL4	HV2 HV1
BL3/HV1	BL3 BL3	BL3 BL3	BL3 BL2	HV1 HV2
BL4/HV1	BL4 BL4	BL4 BL4	BL4 BL3	HV1 HV2

BL - Biosafety Level

HV - Host-Vector System

**Appendix G-III. Footnotes and References of Appendix G**

**Appendix G-III-A.** *Biosafety in Microbiological and Biomedical Laboratories*, 5<sup>th</sup> edition, 2007, DHHS, Public Health Service, [Centers for Disease Control and Prevention](#), Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland.

**Appendix G-III-B.** *Biosafety in Microbiological and Biomedical Laboratories*, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

**Appendix G-III-C.** *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

**Appendix G-III-D.** *National Institutes of Health Biohazards Safety Guide*, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740-00383, 1974.

**Appendix G-III-E.** A. Hellman, M. N. Oxman, and R. Pollack (eds.), *Biohazards in Biological Research*, Cold Spring Harbor Laboratory 1973.

**Appendix G-III-F.** N. V. Steere (ed.), *Handbook of Laboratory Safety*, 2nd edition, The Chemical Rubber Co., Cleveland, Ohio, 1971.

**Appendix G-III-G.** Bodily, J. L., "General Administration of the Laboratory," H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), *Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections*, American Public Health Association, New York, 1970, pp. 11-28.

**Appendix G-III-H.** Darlow, H. M. (1969). "Safety in the Microbiological Laboratory," in J. R. Norris and D. W. Robbins (eds.), *Methods in Microbiology*, Academic Press, Inc., New York, pp. 169-204.

**Appendix G-III-I.** *The Prevention of Laboratory Acquired Infection*, C. H. Collins, E. G. Hartley, and R. Pilsworth, Public Health Laboratory Service, Monograph Series No. 6, 1974.

**Appendix G-III-J.** Chatigny, M. A., "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W. W. Umbreit (ed.), *Advances in Applied Microbiology*, Academic Press, New York, New York, 1961, 3:131-192.

**Appendix G-III-K.** Horsfall, F. L. Jr., and J. H. Baner, *Individual Isolation of Infected Animals in a Single Room*, J. Bact., 1940, 40, 569-580.

**Appendix G-III-L.** Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry, Ann Arbor, Michigan.

**Appendix G-III-M.** Biosafety Level 1 is suitable for work involving agents of unknown or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see [Appendix G-III-A, Footnotes and References of Appendix G](#)).

**Appendix G-III-N.** Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see [Appendix G-III-A, Footnotes and References of Appendix G](#)).

**Appendix G-III-O.** Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, *Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research*, NIH, Bethesda, Maryland 1978.

**Appendix G-III-P.** Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the Principal Investigator.

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## APPENDIX H. SHIPMENT

Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the U.S. Postal Service (39 Code of Federal Regulations, Part 3); the Public Health Service (42 Code of Federal Regulations, Part 72); the U.S. Department of Agriculture (9 Code of Federal Regulations, Subchapters D and E; 7 CFR, Part 340); and/or the U.S. Department of Transportation (49 Code of Federal Regulations, Parts 171-179).

**Note.** A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II, *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under [Section IV-D, Voluntary Compliance](#), will be issued only after consultation with the institution as to the content of the notice (see [Section IV-D-3, Certification of Host-Vector Systems - Voluntary Compliance](#)).

**Appendix H-I.** Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant DNA, if they are regulated as human pathogens by the Public Health Service (42 Code of Federal Regulations, Part 72) or as animal pathogens or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

**Appendix H-II.** Host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA when: (i) the recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest; or (ii) the recombinant DNA codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or (iii) the recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

### **Appendix H-III. Footnotes and References of Appendix H**

For further information on shipping etiologic agents contact: (i) The [Centers for Disease Control and Prevention](#), ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883; (ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or (iii) U.S. Department of Agriculture, ATTN: [Animal and Plant Health Inspection Service \(APHIS\)](#), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

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## **APPENDIX I. BIOLOGICAL CONTAINMENT (See [Appendix E](#), *Certified Host-Vector Systems*)**

### **Appendix I-I. Levels of Biological Containment**

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established. Appendices I-I-A through I-II-B describe levels of biological containment (host-vector systems) for prokaryotes. Specific criteria will depend on the organisms to be used.

#### **Appendix I-I-A. Host-Vector 1 Systems**

Host-Vector 1 systems provide a moderate level of containment. Specific Host-Vector 1 systems are:

##### **Appendix I-I-A-1. *Escherichia coli* K-12 Host-Vector 1 Systems (EK1)**

The host is always *Escherichia coli* K-12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof (see Appendices I-III-A through G, *Footnotes and References of Appendix I*) and variants of bacteriophage, such as lambda (see Appendices I-III-H through O, *Footnotes and References of Appendix I*). The *Escherichia coli* K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

##### **Appendix I-I-A-2. Other Host-Vector 1 Systems**

At a minimum, hosts and vectors shall be comparable in containment to *Escherichia coli* K-12 with a non-conjugative plasmid or bacteriophage vector. [Appendix I-II, Certification of Host-Vector Systems](#), describes the data to be considered and mechanism for approval of Host-Vector 1 systems.

##### **Appendix I-I-B. Host-Vector 2 Systems (EK2)**

Host-Vector 2 Systems provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be  $< 1/10^8$  under specified conditions. Specific Host-Vector 2 systems are:



**Appendix I-I-B-1.** For *Escherichia coli* K-12 Host-Vector 2 systems (EK2) in which the vector is a plasmid, no more than  $1/10^8$  host cells shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

**Appendix I-I-B-2.** For *Escherichia coli* K-12 Host-Vector 2 systems (EK2) in which the vector is a phage, no more than  $1/10^8$  phage particles shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation, or survival in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

## **Appendix I-II. Certification of Host-Vector Systems**

### **Appendix I-II-A. Responsibility**

Host-Vector 1 systems (other than *Escherichia coli* K-12) and Host-Vector 2 systems may not be designated as such until they have been certified by the NIH Director. Requests for certification of host-vector systems may be submitted 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). Proposed host-vector systems will be reviewed by the RAC (see [Section IV-C-1-b-\(1\)-\(f\)](#), *Major Actions*). Initial review will be based on the construction, properties, and testing of the proposed host-vector system by a subcommittee composed of one or more RAC members and/or *ad hoc* experts. The RAC will evaluate the subcommittee's report and any other available information at the next scheduled RAC meeting. The NIH Director is responsible for certification of host-vector systems, following advice of the RAC. Minor modifications to existing host-vector systems (i.e., those that are of minimal or no consequence to the properties relevant to containment) may be certified by the NIH Director without prior RAC review (see [Section IV-C-1-b-\(2\)-\(f\)](#), *Minor Actions*). Once a host-vector system has been certified by the NIH Director, a notice of certification will be sent by NIH/OBA to the applicant and to the Institutional Biosafety Committee Chairs. A list of all currently certified host-vector systems is available 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). The NIH Director may rescind the certification of a host-vector system (see [Section IV-C-1-b-\(2\)-\(g\)](#), *Minor Actions*). If certification is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher level of physical containment level, unless NIH determines that the already constructed clones incorporate adequate biological containment. Certification of an host-vector system does not extend to modifications of either the host or vector component of that system. Such modified systems shall be independently certified by the NIH Director. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications to a certified host-vector system requires submission of complete testing data.

### **Appendix I-II-B. Data to be Submitted for Certification**

#### **Appendix I-II-B-1. Host-Vector 1 Systems Other than *Escherichia coli* K-12**

The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) a description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction, survival, and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and the type of information is exchanged; and any relevant information about its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an Host-Vector 1 system.

## Appendix I-II-B-2. Host-Vector 2 Systems

Investigators planning to request Host-Vector 2 systems certification may obtain instructions from NIH/OBA concerning data to be submitted (see [Appendices I-III-N and O](#), *Footnotes and References of Appendix I*). In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation; and (vi) in some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals or one or more human subjects. Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments. Data shall be submitted 12 weeks prior to the RAC meeting at which such data will be considered by 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). Investigators are encouraged to publish their data on the construction, properties, and testing of proposed Host Vector 2 systems prior to consideration of the system by the RAC and its subcommittee. Specific instructions concerning the submission of data for proposed *Escherichia coli* K-12 Host-Vector 2 system (EK2) involving either plasmids or bacteriophage in *Escherichia coli* K-12, are available 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).

## Appendix I-III. Footnotes and References of Appendix I

**Appendix I-III-A.** Hersfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski, *Plasmid Co1E1 as a Molecular Vehicle for Cloning and Amplification of DNA*. Proc. Nat. Acad. Sci., 1974, 71, pp. 3455-3459.

**Appendix I-III-B.** Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness, *A System for Mapping DNA Sequences in the Chromosomes of Drosophila Melanogaster*. Cell, 1974, 3, pp. 315-335.

**Appendix I-III-C.** Tanaka, T., and B. Weisblum, *Construction of a Colicin EI-R Factor Composite Plasmid in Vitro: Means for Amplification of Deoxyribonucleic Acid*. J. Bacteriol., 1975, 121, pp. 354-362.

**Appendix I-III-D.** Armstrong, K. A., V. Hersfield, and D. R. Helinski, *Gene Cloning and Containment Properties of Plasmid Col E1 and Its Derivatives*, Science, 1977, 196, pp. 172-174.

**Appendix I-III-E.** Bolivar, F., R. L. Rodriguez, M. C. Betlack, and H. W. Boyer, *Construction and Characterization of New Cloning Vehicles: I. Ampicillin-Resistant Derivative of PMB9*, Gene, 1977, 2, pp. 75-93.

**Appendix I-III-F.** Cohen, S. N., A. C. W. Chang, H. Boyer, and R. Helling. *Construction of Biologically Functional Bacterial Plasmids in Vitro*. Proc. Natl. Acad. Sci., 1973, 70, pp. 3240-3244.

**Appendix I-III-G.** Bolivar, F., R. L. Rodriguez, R. J. Greene, M. C. Batlack, H. L. Reyneker, H. W. Boyer, J. H. Cross, and S. Falkow, 1977, *Construction and Characterization of New Cloning Vehicles II. A Multi-Purpose Cloning System*, Gene, 1977, 2, pp. 95-113.

**Appendix I-III-H.** Thomas, M., J. R. Cameron, and R. W. Davis (1974). *Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA*. Proc. Nat. Acad. Sci., 1974, 71, pp. 4579-4583.

**Appendix I-III-I.** Murray, N. E., and K. Murray, *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments*. Nature, 1974, 51, pp. 476-481.

**Appendix I-III-J.** Ramback, A., and P. Tiollais (1974). *Bacteriophage Having EcoRI Endonuclease Sites Only in the Non-Essential Region of the Genome*. Proc. Nat. Acad. Sci., 1974, 71, pp. 3927-3820.



**Appendix I-III-K.** Blattner, F. R., B. G. Williams, A. E. Bleche, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Gunwald, D. O. Kiefer, D. D. Moore, J. W. Shumm, E. L. Sheldon, and O. Smithies, *Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning*, Science 1977, 196, pp. 163-169.

**Appendix I-III-L.** Donoghue, D. J., and P. A. Sharp, *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance*, Gene, 1977, 1, pp. 209-227.

**Appendix I-III-M.** Leder, P., D. Tiemeier and L. Enquist (1977), *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The  $\lambda$ gt WES System*, Science, 1977, 196, pp. 175-177.

**Appendix I-III-N.** Skalka, A., *Current Status of Coliphage AEK2 Vectors*, Gene, 1978, 3, pp. 29-35.

**Appendix I-III-O.** Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein, *Standardized Laboratory Tests for EK2 Certification*, Gene, 1978, 3, pp. 36-38.

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**APPENDIX J. BIOTECHNOLOGY RESEARCH SUBCOMMITTEE**

The National Science and Technology Council's Committee on Fundamental Science determined that a subcommittee should be continued to identify and coordinate Federal research efforts, identify research needs, stimulating international cooperation, and assess national and international policy issues concerning biotechnology sciences. The primary emphasis will be on scientific issues to increase the overall effectiveness and productivity of the Federal investment in biotechnology sciences, especially regarding issues which cut across agency boundaries. This subcommittee is called the Biotechnology Research Subcommittee.

Membership of the Biotechnology Research Subcommittee will include Federal agencies that support biotechnology research. Agencies represented are: U.S. Department of Agriculture, Department of Commerce, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Interior, Department of Justice, Department of State, Department of Veterans Affairs, Agency for International Development, Environmental Protection Agency, National Aeronautics and Space Administration, and National Science Foundation. The Biotechnology Research Subcommittee will function in an advisory capacity to the Committee on Fundamental Science, the Director of the Office of Science and Technology Policy, and the Executive Office of the President. The Biotechnology Research Subcommittee will review the scientific aspects of proposed regulations and guidelines as they are developed.

The primary responsibilities of the Biotechnology Research Subcommittee are to: (i) describe and review current Federal efforts in biotechnology research; (ii) identify and define the priority areas for future Federal biotechnology research, including areas needing greater emphasis, describing the role of each agency in those areas, and delineate where interagency cooperation would enhance progress in the biotechnology sciences, with an emphasis on integrated research efforts, where appropriate; (iii) assess major international efforts in the biotechnology sciences and develop mechanisms for international collaboration. For example, activities of the U.S.-European Community Task Force on Biotechnology have been coordinated through the Biotechnology Research Subcommittee; (iv) identify and review national and international policy issues (such as public education) associated with biotechnology; and (v) provide reviews, analyses, and recommendations to the Chairs of the Committee on Fundamental Science on scientific issues related to regulations and the applications of biotechnology research and biotechnology policies and issues.

In 1990, the Biotechnology Research Subcommittee replaced the Biotechnology Sciences Coordinating Committee. Both the Biotechnology Research Subcommittee and the Biotechnology Sciences Coordinating Committee previously functioned under the Federal Coordinating Council on Science, Engineering, and Technology (FCCSET). While regulatory issues became the primary focus of the Biotechnology Sciences Coordinating Committee, the Biotechnology Research Subcommittee focuses on scientific issues, although it will still provide scientific support for regulatory responsibilities.

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## **APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES**

Appendix K specifies physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to large-scale research or production activities as specified in [Section III-D-6, \*Experiments Involving More than 10 Liters of Culture\*](#). It is important to note that this appendix addresses only the biological hazard associated with organisms containing recombinant DNA. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this appendix.

All provisions shall apply to large-scale research or production activities with the following modifications: (i) Appendix K shall supersede [Appendix G, \*Physical Containment\*](#), when quantities in excess of 10 liters of culture are involved in research or production. [Appendix K-II](#) applies to Good Large Scale Practice; (ii) the institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the Biological Safety Officer shall include those specified in [Section IV-B-3, \*Biological Safety Officer\*](#); (iii) the institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require Biosafety Level (BL) 3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance, and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual, or extended illnesses of employees to determine possible occupational origin.

### **Appendix K-I. Selection of Physical Containment Levels**

The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in [Section III, \*Experiments Covered by the NIH Guidelines\*](#). For purposes of large-scale research or production, four physical containment levels are established. The four levels set containment conditions at those appropriate for the degree of hazard to health or the environment posed by the organism, judged by experience with similar organisms unmodified by recombinant DNA techniques and consistent with Good Large Scale Practice. The four biosafety levels of large-scale physical containment are referred to as Good Large Scale Practice, BL1-Large Scale, BL2-Large Scale, and BL3-Large Scale. Good Large Scale Practice is recommended for large-scale research or production involving viable, non-pathogenic, and non-toxigenic recombinant strains derived from host organisms that have an extended history of safe large-scale use. Good Large Scale Practice is recommended for organisms such as those included in [Appendix C, \*Exemptions under Section III-F-6\*](#), which have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment. BL1-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale and that do not qualify for Good Large Scale Practice. BL2-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL2 containment at the laboratory scale. BL3-Large Scale is recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL3 containment at the laboratory scale. No provisions are made for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

### **Appendix K-II. Good Large Scale Practice (GLSP)**

**Appendix K-II-A.** Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters.

**Appendix K-II-B.** Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant DNA molecules are handled prudently and that the work place is kept clean and orderly.

**Appendix K-II-C.** In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules. Eating, drinking, smoking, applying cosmetics, and mouth pipetting shall be prohibited in the work area.

**Appendix K-II-D.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment.

**Appendix K-II-E.** Discharges containing viable recombinant organisms shall be handled in accordance with applicable governmental environmental regulations.

**Appendix K-II-F.** Addition of materials to a system, sample collection, transfer of culture fluids within/between systems, and processing of culture fluids shall be conducted in a manner that maintains employee's exposure to viable organisms containing recombinant DNA molecules at a level that does not adversely affect the health and safety of employees.

**Appendix K-II-G.** The facility's emergency response plan shall include provisions for handling spills.

### **Appendix K-III. Biosafety Level 1 (BL1) - Large Scale**

**Appendix K-III-A.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Laboratory Director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix K-III-B.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in [Appendix G-II-A, Physical Containment Levels--Biosafety Level 1](#), are met.

**Appendix K-III-C.** Culture fluids (except as allowed in Appendix K-III-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

**Appendix K-III-D.** Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

**Appendix K-III-E.** Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing recombinant DNA molecules to the environment.

**Appendix K-III-F.** A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in [Appendix K-III-C](#) above. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

**Appendix K-III-G.** Emergency plans required by [Sections IV-B-2-b-\(6\)](#), *Institutional Biosafety Committee*, and [IV-B-3-c-\(3\)](#), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

## **Appendix K-IV. Biosafety Level 2 (BL2) - Large Scale**

**Appendix K-IV-A.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent 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). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix K-IV-B.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in [Appendix G-II-B, Physical Containment Levels--Biosafety Level 2](#), are met.

**Appendix K-IV-C.** Culture fluids (except as allowed in Appendix K-IV-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

**Appendix K-IV-D.** Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

**Appendix K-IV-E.** Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

**Appendix K-IV-F.** A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in [Appendix K-IV-C](#) above. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

**Appendix K-IV-G.** Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

**Appendix K-IV-H.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing sensing devices that monitor the integrity of containment during operations.

**Appendix K-IV-I.** A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

**Appendix K-IV-J.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

**Appendix K-IV-K.** The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

**Appendix K-IV-L.** Emergency plans required by [Sections IV-B-2-b-\(6\)](#), *Institutional Biosafety Committee*, and [IV-B-3-c-\(3\)](#), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

### **Appendix K-V. Biosafety Level 3 (BL3) - Large Scale**

**Appendix K-V-A.** Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent 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). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

**Appendix K-V-B.** Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in [Appendix G-II-C](#), *Physical Containment Levels--Biosafety Level 3*, are met.

**Appendix K-V-C.** Culture fluids (except as allowed in Appendix K-V-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

**Appendix K-V-D.** Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

**Appendix K-V-E.** Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

**Appendix K-V-F.** A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure except when the culture fluids contain viable organisms or vectors intended as final product as described in [Appendix K-V-C](#) above. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

**Appendix K-V-G.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.

**Appendix K-V-H.** Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

**Appendix K-V-I.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

**Appendix K-V-J.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

**Appendix K-V-K.** A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, maintenance, and use of this equipment for research production activities involving viable organisms containing recombinant DNA molecules.

**Appendix K-V-L.** The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

**Appendix K-V-M.** Emergency plans required by [Sections IV-B-2-b-\(6\)](#), *Institutional Biosafety Committee*, and [IV-B-3-c-\(3\)](#), *Biological Safety Officer*, shall include methods and procedures for handling large losses of culture on an emergency basis.

**Appendix K-V-N.** Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:

**Appendix K-V-N-1.** The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.

**Appendix K-V-N-2.** The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

**Appendix K-V-N-3.** Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

**Appendix K-V-N-4.** All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

**Appendix K-V-N-5.** Hand washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.

**Appendix K-V-N-6.** A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

**Appendix K-V-N-7.** The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.



**Appendix K-V-N-8.** The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may not be discharged to the outdoors without being high efficiency particulate air/HEPA filtered, subjected to thermal oxidation, or otherwise treated to prevent the release of viable organisms.

**Appendix K-V-O.** The following personnel and operational practices shall be required:

**Appendix K-V-O-1.** Personnel entry into the controlled area shall be through the entry area specified in Appendix K-V-N-1.

**Appendix K-V-O-2.** Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

**Appendix K-V-O-3.** Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

**Appendix K-V-O-4.** Persons under 18 years of age shall not be permitted to enter the controlled area.

**Appendix K-V-O-5.** The universal biosafety sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

**Appendix K-V-O-6.** The controlled area shall be kept neat and clean.

**Appendix K-V-O-7.** Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

**Appendix K-V-O-8.** Animals and plants shall be excluded from the controlled area.

**Appendix K-V-O-9.** An effective insect and rodent control program shall be maintained.

**Appendix K-V-O-10.** Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Service doors leading directly outdoors shall be sealed and locked while work is in progress.

**Appendix K-V-O-11.** Persons shall wash their hands when exiting the controlled area.

**Appendix K-V-O-12.** Persons working in the controlled area shall be trained in emergency procedures.

**Appendix K-V-O-13.** Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

**Appendix K-V-O-14.** The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

**Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL) - Large Scale (LS) Practice** (See [Appendix K-VI-A](#), *Footnotes Of Appendix K*)

CRITERION [See <a href="#">Appendix K-VI-B</a> , <i>Footnotes of Appendix K</i> ]		GLSP	BL1-LS	BL2-LS	BL3-LS
1.	Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures.	K-II-A	G-I		
2.	Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees.	K-II-B	G-I		
3.	Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work.	K-II-C	G-II-A-1-h	G-II-B-2-f	G-II-C-2-i
4.	Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place.	K-II-C	G-II-A-1-d G-II-A-1-e	G-II-B-1-d G-II-B-1-e	G-II-C-1-c G-II-C-1-d
5.	Internal accident reporting.	K-II-G	K-III-A	K-IV-A	K-V-A
6.	Medical surveillance.	NR	NR		
7.	Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment).	NR	K-III-B	K-IV-B	K-V-B
8.	Culture fluids not removed from a system until organisms are inactivated.	NR	K-III-C	K-IV-C	K-V-C
9.	Inactivation of waste solutions and materials with respect to their biohazard potential.	K-II-E	K-III-C	K-IV-C	K-V-C
10.	Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system.	Minimize <i>Procedure</i> K-II-F	Minimize <i>Engineer</i> K-III-B K-III-D	Prevent <i>Engineer</i> K-IV-B K-IV-D	Prevent <i>Engineer</i> K-V-B K-V-D
11.	Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.	NR	Minimize K-III-E	Prevent K-IV-E	Prevent K-V-E
12.	Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure.	NR	K-III-F	K-IV-F	K-V-F
13.	Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features.	NR	NR	NR	K-V-G
14.	Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.	NR	NR	Prevent K-IV-G	Prevent K-V-H
15.	Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.	NR	NR	K-IV-H	K-V-I
16.	Validated integrity testing of closed containment system.	NR	NR	K-IV-I	K-V-J
17.	Closed system to be permanently identified for record keeping purposes.	NR	NR	K-IV-J	K-V-K
18.	Universal biosafety sign to be posted on each closed system.	NR	NR	K-IV-K	K-V-L
19.	Emergency plans required for handling large losses of cultures.	K-II-G	K-III-G	K-IV-L	K-V-M
20.	Access to the work place.	NR	G-II-A-1-a	G-II-B-1-a	K-V-N
21.	Requirements for controlled access area.	NR	NR	NR	K-V-N&O

NR = not required



## Appendix K-VI. Footnotes of Appendix K

**Appendix K-VI-A.** This table is derived from the text in Appendices G (*Physical Containment*) and K and is not to be used in lieu of Appendices G and K.

**Appendix K-VI-B.** The criteria in this grid address only the biological hazards associated with organisms containing recombinant DNA. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this grid.

## Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K

**Appendix K-VII-A. Accidental Release.** An accidental release is the unintentional discharge of a microbiological agent (i.e., microorganism or virus) or eukaryotic cell due to a failure in the containment system.

**Appendix K-VII-B. Biological Barrier.** A biological barrier is an impediment (naturally occurring or introduced) to the infectivity and/or survival of a microbiological agent or eukaryotic cell once it has been released into the environment.

**Appendix K-VII-C. Closed System.** A closed system is one in which by its design and proper operation, prevents release of a microbiological agent or eukaryotic cell contained therein.

**Appendix K-VII-D. Containment.** Containment is the confinement of a microbiological agent or eukaryotic cell that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include: physical and biological barriers and inactivation using physical or chemical means.

**Appendix K-VII-E. De minimis Release.** *De minimis* release is the release of: (i) viable microbiological agents or eukaryotic cells that does not result in the establishment of disease in healthy people, plants, or animals; or (ii) in uncontrolled proliferation of any microbiological agents or eukaryotic cells.

**Appendix K-VII-F. Disinfection.** Disinfection is a process by which viable microbiological agents or eukaryotic cells are reduced to a level unlikely to produce disease in healthy people, plants, or animals.

**Appendix K-VII-G. Good Large Scale Practice Organism.** For an organism to qualify for Good Large Scale Practice consideration, it must meet the following criteria [Reference: Organization for Economic Cooperation and Development, *Recombinant DNA Safety Considerations*, 1987, p. 34-35]: (i) the host organism should be non-pathogenic, should not contain adventitious agents and should have an extended history of safe large-scale use or have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment; (ii) the recombinant DNA-engineered organism should be non-pathogenic, should be as safe in the large-scale setting as the host organism, and without adverse consequences in the environment; and (iii) the vector/insert should be well characterized and free from known harmful sequences; should be limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the construct in the environment unless that is a requirement of the intended function; should be poorly mobilizable; and should not transfer any resistance markers to microorganisms unknown to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

**Appendix K-VII-H. Inactivation.** Inactivation is any process that destroys the ability of a specific microbiological agent or eukaryotic cell to self-replicate.

**Appendix K-VII-I. Incidental Release.** An incidental release is the discharge of a microbiological agent or eukaryotic cell from a containment system that is expected when the system is appropriately designed and properly operated and maintained.

**Appendix K-VII-J. Minimization.** Minimization is the design and operation of containment systems in order that any incidental release is a *de minimis* release.

**Appendix K-VII-K. Pathogen.** A pathogen is any microbiological agent or eukaryotic cell containing sufficient genetic information, which upon expression of such information, is capable of producing disease in healthy people, plants, or animals.

**Appendix K-VII-L. Physical Barrier.** A physical barrier is considered any equipment, facilities, or devices (e.g., fermentors, factories, filters, thermal oxidizers) which are designed to achieve containment.

**Appendix K-VII-M. Release.** Release is the discharge of a microbiological agent or eukaryotic cell from a containment system. Discharges can be incidental or accidental. Incidental releases are *de minimis* in nature; accidental releases may be *de minimis* in nature.

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**APPENDIX L. GENE THERAPY POLICY CONFERENCES (GTPCS)**

In order to enhance the depth and value of public discussion relevant to scientific, safety, social, and ethical implications of gene therapy research, the NIH Director will convene GTPCs at regular intervals. As appropriate, the NIH Director may convene a GTPC in conjunction with a RAC meeting. GTPCs will be administered by NIH/OBA. Conference participation will not involve a standing committee membership but rather will offer the unique advantage of assembling numerous participants who possess significant scientific, ethical, and legal expertise and/or interest that is directly applicable to a specific gene therapy research issue. At least one member of RAC will serve as Co-chair of each GTPC and report the findings of each GTPC to RAC at its next scheduled meeting. The RAC representative for each GTPC will be chosen based on the participant's area of expertise relative to the specific gene therapy research issue to be discussed. All RAC members will be invited to attend GTPCs. GTPCs will have representation from other Federal agencies, including FDA and OHRP. GTPCs will focus on broad overarching policy and scientific issues related to gene therapy research. Proposals for GTPC topics may be submitted by members of RAC, representatives of academia, industry, patient and consumer advocacy organizations, other Federal agencies, professional scientific societies, and the general public. GTPC topics will not be limited to discussion of human applications of gene therapy research, i.e., they may include basic research on the use of novel gene delivery vehicles, or novel applications of human gene transfer. The RAC, with the Director's approval, will have the primary responsibility for planning GTPC agendas. GTPC findings will be transmitted to the NIH Director and will be made publicly available. The NIH Director anticipates that this public policy forum will serve as a model for interagency communication and collaboration, concentrated expert discussion of novel scientific issues and their potential societal implications, and enhanced opportunity for public discussion of specific issues and potential impact of such applications on human health and the environment.

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**APPENDIX M. POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO ONE OR MORE HUMAN RESEARCH PARTICIPANTS (POINTS TO CONSIDER)**

Appendix M applies to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH. Researchers not covered by the *NIH Guidelines* are encouraged to use Appendix M (see [Section I-C, General Applicability](#)).

The acceptability of human somatic cell gene transfer has been addressed in several public documents as well as in numerous academic studies. In November 1982, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research published a report, *Splicing Life*, which resulted from a two-year process of public deliberation and hearings. Upon release of that report, a U.S. House of Representatives subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, *Human Gene Therapy*, which concluded that civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene transfer of somatic cells in humans for specific genetic diseases. Somatic cell gene transfer is seen as an extension of present methods that might be preferable to other technologies. In light of this public support, RAC is prepared to consider proposals for somatic cell gene transfer.

RAC will not at present entertain proposals for germ line alterations but will consider proposals involving somatic cell gene transfer. The purpose of somatic cell gene transfer is to treat an individual patient, e.g., by inserting a properly functioning gene into the subject's somatic cells. Germ line alteration involves a specific attempt to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring.

The RAC continues to explore the issues raised by the potential of *in utero* gene transfer clinical research. However, the RAC concludes that, at present, it is premature to undertake any *in utero* gene transfer clinical trial. Significant additional preclinical and clinical studies addressing vector transduction efficacy, biodistribution, and toxicity are required before a human *in utero* gene transfer protocol can proceed. In addition, a more thorough understanding of the development of human organ systems, such as the immune and nervous systems, is needed to better define the potential efficacy and risks of human *in utero* gene transfer. Prerequisites for considering any specific human *in utero* gene transfer procedure include an understanding of the pathophysiology of the candidate disease and a demonstrable advantage to the *in utero* approach. Once the above criteria are met, the RAC would be willing to consider well rationalized human *in utero* gene transfer clinical trials.

Research proposals involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer) will be considered through a review process involving both NIH/OBA and RAC. Investigators shall submit their relevant information on the proposed human gene transfer experiments to NIH/OBA. Submission of human gene transfer protocols to NIH will be in the format described in [Appendix M-I-A, Submission Requirements for Protocol Submission](#). Submission to NIH shall be for registration purposes and will ensure continued public access to relevant human gene transfer information conducted in compliance with the *NIH Guidelines*. Investigational New Drug (IND) applications should be submitted to [FDA](#) in the format described in 21 CFR, Chapter I, Subchapter D, Part 312, Subpart B, Section 23, *IND Content and Format*.

Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

Factors that may contribute to public discussion of a human gene transfer experiment by RAC include: (i) new vectors/new gene delivery systems, (ii) new diseases, (iii) unique applications of gene transfer, and (iv) other issues considered to require further public discussion. Among the experiments that may be considered exempt from RAC discussion are those determined not to represent possible risk to human health or the environment. Full, public RAC review and discussion of a human gene transfer experiment may be (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members, or (b) a Federal agency other than NIH. An individual human gene transfer experiment that is recommended for full RAC review should represent novel characteristics deserving of public discussion. If it is determined that an experiment will undergo full RAC discussion, NIH/OBA will immediately notify the Principal Investigator. RAC members may forward individual requests for additional information relevant to a specific protocol through NIH/OBA to the Principal Investigator. In making a determination whether an experiment is novel, and thus deserving of full RAC discussion, reviewers will examine the scientific rationale, scientific context (relative to other proposals reviewed by RAC), whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. RAC recommendations on a specific human gene transfer experiment shall be forwarded to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate. Relevant documentation will be included in the material for the RAC meeting at which the experiment is scheduled to be discussed. RAC meetings will be open to the public except where trade secrets and proprietary information are reviewed (see [Section IV-D-5, Protection of Proprietary Data – Voluntary Compliance](#)). RAC prefers that information provided in response to Appendix M contain no proprietary data or trade secrets, enabling all aspects of the review to be open to the public.

**Note:** Any application submitted to NIH/OBA shall not be designated as 'confidential' in its entirety. In the event that a sponsor determines that specific responses to one or more of the items described in Appendix M should be considered as proprietary or trade secret, each item should be clearly identified as such. The cover letter (attached to the submitted material) shall: (1) clearly indicate that select portions of the application contain information considered as proprietary or trade secret, (2) a brief explanation as to the reason that each of these items is determined proprietary or trade secret.

Public discussion of human gene transfer experiments (and access to relevant information) shall serve to inform the public about the technical aspects of the proposals, meaning and significance of the research, and significant safety, social, and ethical implications of the research. RAC discussion is intended to ensure safe and ethical conduct of gene transfer experiments and facilitate public understanding of this novel area of biomedical research.

In its evaluation of human gene transfer proposals, RAC will consider whether the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of clinical investigation, namely, to protect the health and well being of human subjects being treated while at the same time gathering generalizable knowledge. Two possible undesirable consequences of the transfer of recombinant DNA would be unintentional: (i) vertical transmission of genetic changes from an individual to his/her offspring, or (ii) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, Appendices M-I through M-V request information that will enable RAC and NIH/OBA to assess the possibility that the proposed experiment(s) will inadvertently affect reproductive cells or lead to infection of other people (e.g., medical personnel or relatives).

Appendix M will be considered for revisions as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out periodically as needed.

## **Appendix M-I. Requirements for Protocol Submission, Review, and Reporting – Human Gene Transfer Experiments**

### **Appendix M-I-A. Requirements for Protocol Submission**

The following documentation must be submitted (see exemption in [Appendix M-VI-A, Footnotes of Appendix M](#)) in printed or electronic form 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), E-mail: [rosenthg@od.nih.gov](mailto:rosenthg@od.nih.gov). NIH OBA will confirm receipt within three working days after receiving the submission. Investigators should contact OBA if they do not receive this confirmation.

1. A cover letter on institutional letterhead, signed by the Principal Investigator(s), that: (1) acknowledges that the documentation submitted to NIH OBA complies with the requirements set forth in Appendix M-I-A, *Requirements for Protocol Submission*; (2) identifies the Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) at the proposed clinical trial site(s) responsible for local review and approval of the protocol; and (3) acknowledges that no research participant will 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](#)); IBC approval (from the clinical trial site) has been obtained; IRB approval has been obtained; and all applicable regulatory authorizations have been obtained.
2. The scientific abstract.
3. The non-technical abstract.
4. The proposed clinical protocol, including tables, figures, and relevant manuscripts.
5. Responses to Appendices M-II through M-V, *Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues*. Responses to Appendices M-II through M-V may be provided either as an appendix to the clinical protocol or incorporated in the clinical protocol. If responses to [Appendices M-II](#) through M-V are incorporated in the clinical protocol, each response must refer to the appropriate Appendix M-II through M-V.

6. The proposed informed consent document (see [Appendix M-III, Informed Consent](#)).
7. Curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format).

**Note:** A human gene transfer experiment submitted to NIH OBA should not contain confidential commercial information or trade secrets, enabling all aspects of the review to be open to the public.

## **Appendix M-I-B. RAC Review Requirements**

### **Appendix M-I-B-1. Initial RAC Review**

The initial RAC review process shall include a determination as to whether the human gene transfer experiment presents characteristics that warrant public RAC review and discussion. During the RAC's initial review, individual committee members may request additional information relevant to the protocol. NIH OBA will immediately notify the Principal Investigator(s) of RAC requests for additional information. In making a determination whether an experiment presents characteristics warranting public RAC review and discussion, reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been resolved. Other factors that may warrant public review and discussion of a human gene transfer experiment by the RAC include: (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion.

Initial RAC review shall be completed within 15 working days of receipt of a complete submission (see [Appendix M-I-A, Requirements for Protocol Submission](#)). At the end of the 15-day review period, NIH OBA will notify the Principal Investigator(s) in writing about the results of the RAC's initial review. Two outcomes are possible: (1) the experiment does not present characteristics that warrant further review and discussion and is therefore exempt from public RAC review and discussion; or (2) the experiment presents characteristics that warrant public RAC review and discussion. Completion of the RAC review process is defined as: (1) receipt by the Principal Investigator(s) of a letter from NIH OBA indicating that the submission does not present characteristics that warrant public RAC review and discussion; or (2) receipt by the Principal Investigator(s) of a letter from NIH OBA after public RAC review that summarizes the committee's key comments and recommendations (if any).

If a human gene transfer protocol is submitted less than eight weeks before a scheduled RAC meeting and is subsequently recommended for public RAC review and discussion, the review of the protocol by the RAC will be deferred until the next scheduled RAC meeting. This eight-week period is needed to ensure adequate time for public notice and comment and thorough review by the committee members.

No research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in the human gene transfer experiment until: (1) the RAC review process has been completed; (2) Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained; (3) Institutional Review Board (IRB) approval has been obtained; and (4) all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant numbers(s) if applicable.

### **Appendix M-I-B-2. Public RAC Review and Discussion**

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. In making a determination whether an experiment presents characteristics warranting public RAC review and discussion, reviewers will examine the scientific rationale, scientific content, whether the preliminary *in vitro* and *in vivo* safety data were obtained in appropriate models and are sufficient, and whether questions related to relevant social and ethical issues have been



resolved. Other factors that may warrant public review and discussion of a human gene transfer experiment by the RAC include: (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion.

After a human gene transfer experiment is reviewed by the full RAC at a regularly scheduled meeting, NIH OBA will send a letter summarizing the RAC key comments and recommendations (if any) regarding the protocol to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate. Completion of RAC review is defined as receipt by the Principal Investigator(s) of a letter from NIH OBA summarizing the committee's findings. Unless NIH OBA determines that there are exceptional circumstances, the RAC summary letter will be sent to the Principal Investigator(s) within 10 working days after the completion of the RAC meeting at which the experiment was reviewed.

RAC meetings will be open to the public except where trade secrets or confidential commercial information are reviewed. To enable all aspects of the protocol review process to be open to the public, information provided in response to Appendix M should not contain trade secrets or confidential commercial information. No application submitted to NIH OBA shall be designated as 'confidential' in its entirety. In the event that an investigator determines that specific responses to one or more of the items described in Appendix M should be considered as confidential commercial information or a trade secret, each item must be clearly identified as such. The cover letter (attached to the submitted material) shall: (1) clearly designate the information that is considered as confidential commercial information or a trade secret; and (2) explain and justify each designation.

## **Appendix M-I-C. Reporting Requirements**

### **Appendix M-I-C-1. Initiation of the Clinical Investigation**

No later than 20 working days after enrollment (see definition of enrollment in [Section I-E-7](#)) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the [FDA Investigational New Drug Application \(IND\)](#) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

### **Appendix M-I-C-2. Additional Clinical Trial Sites**

No research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

### **Appendix M-I-C-3. Annual Reports**

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.

(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number

whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

#### **Appendix M-I-C-4. Safety Reporting**

Principal Investigators must submit, in accordance with this section, Appendix M-I-C-4-a and [Appendix M-I-C-4-b](#), a written report on: (1) any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events); and (2) any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in [Appendix M-I-C-4-b](#).

Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Principal Investigators may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone and fax numbers of the contact(s). The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

The three alternative mechanisms for reporting serious adverse events to the NIH OBA are: by e-mail to [oba@od.nih.gov](mailto:oba@od.nih.gov); by fax to 301-496-9839; or by mail to the Office of Biotechnology Activities, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985.

#### **Appendix M-I-C-4-a. Safety Reporting: Content and Format**

The serious adverse event report must include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type, e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo*, *ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at: [http://oba.od.nih.gov/rdna/adverse\\_event\\_oba.html](http://oba.od.nih.gov/rdna/adverse_event_oba.html), the [FDA MedWatch](#) forms, or other means provided that all of the above elements are specifically included.

Reports from laboratory animal studies as delineated in [Appendix M-I-C-4](#) must be submitted in a narrative format.

**Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports**

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Changes in this schedule are permitted only where, under the FDA IND regulations [21 CFR 312(c)(3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity must be reported as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

**Appendix M-I-C-5. Confidentiality**

Data submitted in accordance with [Appendix M-I-C](#) that are claimed to be confidential commercial or trade secret information must be clearly labeled as such. Prior to making its determination about the confidentiality of data labeled confidential commercial or trade secret, the NIH will contact the Principal Investigator or delegate to ascertain the basis for the claim and subsequently will notify the Principal Investigator or delegate of its final determination regarding the claim.

If NIH determines that the data so labeled are confidential commercial or trade secret and that their public disclosure would promote an understanding of key scientific or safety issues, the NIH will seek agreement from the appropriate party to release such data. Public discussion of scientific and safety issues raised by data submitted in accordance with [Appendix M-I-C](#) is vital to informing both investigators and human subjects about the safety of gene transfer research.

To protect the privacy of participants in gene transfer research, any serious adverse event or annual reports submitted to NIH OBA must not contain any information that would identify the human research participants.

**Appendix M-I-D. Safety Assessment in Human Gene Transfer Research**

A working group of the RAC, the NIH Gene Transfer Safety Assessment Board, with staff support from the NIH OBA, will: 1) review in closed session as appropriate safety information from gene transfer trials for the purpose of assessing toxicity and safety data across gene transfer trials; 2) identify significant trends or significant single events; and 3) report significant findings and aggregated trend data to the RAC. It is expected that this process will enhance review of new protocols, improve the development, design, and conduct of human gene transfer trials, promote public understanding and awareness of the safety of human gene transfer research studies, and inform the decision-making of potential trial participants.



## **Appendix M-II. Description of the Proposal**

Responses to this appendix should be provided in the form of either written answers or references to specific sections of the protocol or its appendices. Investigators should indicate the points that are not applicable with a brief explanation. Investigators submitting proposals that employ the same vector systems may refer to preceding documents relating to the vector sequence without having to rewrite such material.

### **Appendix M-II-A. Objectives and Rationale of the Proposed Research**

State concisely the overall objectives and rationale of the proposed study. Provide information on the specific points that relate to whichever type of research is being proposed.

#### **Appendix M-II-A-1. Use of Recombinant DNA for Therapeutic Purposes**

For research in which recombinant DNA is transferred in order to treat a disease or disorder (e.g., genetic diseases, cancer, and metabolic diseases), the following questions should be addressed:

**Appendix M-II-A-1-a.** Why is the disease selected for experimental treatment by means of gene transfer a good candidate for such treatment?

**Appendix M-II-A-1-b.** Describe the natural history and range of expression of the disease selected for experimental treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene transfer?

**Appendix M-II-A-1-c.** Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

**Appendix M-II-A-1-d.** What alternative therapies exist? In what groups of subjects are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene transfer?

#### **Appendix M-II-A-2. Transfer of DNA for Other Purposes**

**Appendix M-II-A-2-a.** Into what cells will the recombinant DNA be transferred? Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using recombinant DNA?

**Appendix M-II-A-2-b.** What alternative methodologies exist? What are their relative advantages and disadvantages as compared to the use of recombinant DNA?

### **Appendix M-II-B. Research Design, Anticipated Risks and Benefits**

#### **Appendix M-II-B-1. Structure and Characteristics of the Biological System**

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

**Appendix M-II-B-1-a.** What is the structure of the cloned DNA that will be used?

**Appendix M-II-B-1-a-(1).** Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

**Appendix M-II-B-1-a-(2).** What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)? From what source are these elements derived? Summarize what is currently known about the regulatory character of each element.

**Appendix M-II-B-1-a-(3).** Describe the steps used to derive the DNA construct.

**Appendix M-II-B-1-b.** What is the structure of the material that will be administered to the research participant?

**Appendix M-II-B-1-b-(1).** Describe the preparation, structure, and composition of the materials that will be given to the human research subject or used to treat the subject's cells: (i) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests? (ii) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or contaminating viruses (both replication-competent or replication-defective) or other organisms in the cells or serum used for preparation of the virus stock including any contaminants that may have biological effects? (iii) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being conducted to assess the material to be returned to the subject for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells? (iv) If methods other than those covered by Appendices M-II-B-1 through M-II-B-3, *Research Design, Anticipated Risks and Benefits*, are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are possible sources of contamination? What is the sensitivity of tests used to monitor contamination?

**Appendix M-II-B-1-b-(2).** Describe any other material to be used in preparation of the material to be administered to the human research subject. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

## **Appendix M-II-B-2. Preclinical Studies, Including Risk-Assessment Studies**

Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.

### **Appendix M-II-B-2-a. Delivery System**

**Appendix M-II-B-2-a-(1).** What cells are the intended target cells of recombinant DNA? What target cells are to be treated *ex vivo* and returned to the human subject, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?

**Appendix M-II-B-2-a-(2).** Is the delivery system efficient? What percentage of the target cells contain the added DNA?

**Appendix M-II-B-2-a-(3).** How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

**Appendix M-II-B-2-a-(4).** How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

### **Appendix M-II-B-2-b. Gene Transfer and Expression**

**Appendix M-II-B-2-b-(1).** What animal and cultured cell models were used in laboratory studies to assess the *in vivo* and *in vitro* efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

**Appendix M-II-B-2-b-(2).** What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

**Appendix M-II-B-2-b-(3).** Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression.

**Appendix M-II-B-2-b-(4).** To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

**Appendix M-II-B-2-b-(5).** In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

**Appendix M-II-B-2-b-(6).** Is the gene expressed in cells other than the target cells? If so, to what extent?

#### **Appendix M-II-B-2-c. Retrovirus Delivery Systems**

**Appendix M-II-B-2-c-(1).** What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

**Appendix M-II-B-2-c-(2).** How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the human subject's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

**Appendix M-II-B-2-c-(3).** What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

**Appendix M-II-B-2-c-(4).** Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of these analyses?

**Appendix M-II-B-2-c-(5).** Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

#### **Appendix M-II-B-2-d. Non-Retrovirus Delivery/Expression Systems**

If a non-retroviral delivery system is used, what animal studies have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells)? How long have the animals been studied after treatment? What safety studies have been conducted? (Include data about the level of sensitivity of such assays.)

#### **Appendix M-II-B-3. Clinical Procedures, Including Research Participant Monitoring**

Describe the experimental treatment that will be administered to the human subjects and the diagnostic methods that will be used to monitor the success or failure of the experimental treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:

**Appendix M-II-B-3-a.** Will cells (e.g., bone marrow cells) be removed from human subjects and treated *ex vivo*? If so, describe the type, number, and intervals at which these cells will be removed.

**Appendix M-II-B-3-b.** Will human subjects be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy)?

**Appendix M-II-B-3-c.** What treated cells (or vector/DNA combination) will be given to human subjects? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple experimental treatments? If so, over what period of time?

**Appendix M-II-B-3-d.** How will it be determined that new gene sequences have been inserted into the subject's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses?

**Appendix M-II-B-3-e.** What studies will be conducted to assess the presence and effects of the contaminants?

**Appendix M-II-B-3-f.** What are the clinical endpoints of the study? Are there objectives and quantitative measurements to assess the natural history of the disease? Will such measurements be used in human subject follow-up? How will subjects be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will follow-up continue?

**Appendix M-II-B-3-g.** What are the major beneficial and adverse effects of the experimental treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used.

**Appendix M-II-B-3-h.** If a treated human subject dies, what special post-mortem studies will be performed?

#### **Appendix M-II-B-4. Public Health Considerations**

Describe any potential benefits and hazards of the proposed gene transfer to persons other than the human subjects receiving the experimental treatment. Specifically:

**Appendix M-II-B-4-a.** On what basis are potential public health benefits or hazards postulated?

**Appendix M-II-B-4-b.** Is there a significant possibility that the added DNA will spread from the human subject to other persons or to the environment?

**Appendix M-II-B-4-c.** What precautions will be taken against such spread (e.g., subjects sharing a room, health-care workers, or family members)?

**Appendix M-II-B-4-d.** What measures will be undertaken to mitigate the risks, if any, to public health?

**Appendix M-II-B-4-e.** In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to subjects? Are such concerns applicable to health care personnel?

#### **Appendix M-II-B-5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities**

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of recombinant DNA. Describe the laboratory and clinical facilities where the proposed study will be performed. Specifically:

**Appendix M-II-B-5-a.** What professional personnel (medical and nonmedical) will be involved in the proposed study and what is their relevant expertise? Provide a two-page curriculum vitae for each key professional person in biographical sketch format (see [Appendix M-I-A, Requirements for Protocol Submission](#)).

**Appendix M-II-B-5-b.** At what hospital or clinic will the experimental treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will subjects occupy regular hospital beds or clinical research center beds? Where will subjects reside during the follow-up period? What special arrangements will be made for the comfort and consideration of the research participants. Will the research institution designate an ombudsman, patient care representative, or other individual to help protect the rights and welfare of the research participant?

#### **Appendix M-II-C. Selection of the Human Subjects**

Estimate the number of human subjects to be involved in the proposed study. Describe recruitment procedures and eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable. Specifically:

**Appendix M-II-C-1.** How many subjects do you plan to involve in the proposed study?

**Appendix M-II-C-2.** How many eligible subjects do you anticipate being able to identify each year?

**Appendix M-II-C-3.** What recruitment procedures do you plan to use?

**Appendix M-II-C-4.** What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

**Appendix M-II-C-5.** How will subjects be selected if it is not possible to include all who desire to participate?

### **Appendix M-III. Informed Consent**

In accordance with the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should indicate how the Informed Consent document makes clear the special requirements of gene transfer research. If a proposal involves children, special attention should be paid to the Protection of Human Subjects (45 CFR Part 46), Subpart D, Additional Protections for Children Involved as Subjects in Research.

#### **Appendix M-III-A. Communication About the Study to Potential Participants**

**Appendix M-III-A-1.** Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?

**Appendix M-III-A-2.** How will the major points covered in [Appendix M-II, Description of Proposal](#), be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?

**Appendix M-III-A-3.** What is the length of time that potential participants will have to make a decision about their participation in the study?

**Appendix M-III-A-4.** If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?

#### **Appendix M-III-B. Informed Consent Document**

Submission of a human gene transfer experiment to NIH OBA must include a copy of the proposed informed consent document. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a "marker" or to enhance the power of immunotherapy for cancer.

Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent document should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45 CFR 46). Indicate if each of the specified items appears in the Informed Consent document or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are omitted from the consent process or the Informed Consent document.

#### **Appendix M-III-B-1. General Requirements of Human Subjects Research**

##### **Appendix M-III-B-1-a. Description/Purpose of the Study**

The subjects should be provided with a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component.

**Appendix M-III-B-1-b. Alternatives**

The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches.

**Appendix M-III-B-1-c. Voluntary Participation**

The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled.

**Appendix M-III-B-1-d. Benefits**

The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others.

**Appendix M-III-B-1-e. Possible Risks, Discomforts, and Side Effects**

There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization.

If verbal descriptors (e.g., "rare," "uncommon," or "frequent") are used to express quantitative information regarding risk, these terms should be explained.

The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe.

The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started.

**Appendix M-III-B-1-f. Costs**

The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved.

Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury.

**Appendix M-III-B-2. Specific Requirements of Gene Transfer Research****Appendix M-III-B-2-a. Reproductive Considerations**

To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified.

The inclusion of pregnant or lactating women should be addressed.

**Appendix M-III-B-2-b. Long-Term Follow-Up**

To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The investigator should request that subjects continue to provide a current address and telephone number.

The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed.

**Appendix M-III-B-2-c. Request for Autopsy**

To obtain vital information about the safety and efficacy of gene transfer, subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance.

**Appendix M-III-B-2-d. Interest of the Media and Others in the Research**

To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (i) that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (ii) that representatives of applicable Federal agencies (e.g., the [National Institutes of Health](#) and the [Food and Drug Administration](#)), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records.

**Appendix M-IV. Privacy**

Indicate what measures will be taken to protect the privacy of subjects and their families as well as maintain the confidentiality of research data. These measures should help protect the confidentiality of information that could directly or indirectly identify study participants.

**Appendix M-IV-A.** What provisions will be made to honor the wishes of individual human subjects (and the parents or guardians of pediatric or mentally handicapped subjects) as to whether, when, or how the identity of a subject is publicly disclosed.

**Appendix M-IV-B.** What provisions will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual subjects?

**Appendix M-V. Special Issues**

Although the following issues are beyond the normal purview of local Institutional Review Boards, investigators should respond to the following questions:

**Appendix M-V-A.** What steps will be taken, consistent with Appendix M-IV, *Privacy*, to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

**Appendix M-V-B.** Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

## Appendix M-VI. Footnotes of Appendix M

**Appendix M-VI-A.** Human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are exempt from [Appendix M-I, Requirements for Protocol Submission, Review and Reporting – Human Gene Transfer Experiments](#).

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## APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the *NIH Guidelines* apply to plant research activities with the following modifications:

Appendix P shall supersede [Appendix G \(Physical Containment\)](#) when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in [Appendix G](#). The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

### Appendix P-I. General Plant Biosafety Levels

**Appendix P-I-A.** The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

**Appendix P-I-B.** The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

**Appendix P-I-C.** Four biosafety levels, referred to as Biosafety Level (BL) 1 - Plants (P), BL2-P, BL3-P, and BL4-P, are established in [Appendix P-II, Physical Containment Levels](#). The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in [Appendix P-III, Biological Containment Practices](#). These biosafety levels are described in [Appendix P-II, Physical Containment Levels](#). This appendix describes greenhouse practices and special greenhouse facilities for physical containment.



**Appendix P-I-D.** BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in [Appendix P-III, \*Biological Containment Practices\*](#), provide flexible approaches to ensure the safe conduct of research.

**Appendix P-I-E.** For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in [Appendix G, \*Physical Containment\*](#). These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see [Appendix P-III, \*Biological Containment Practices\*](#)), if botanical reproductive structures are produced that have the potential of being released.

## **Appendix P-II. Physical Containment Levels**

### **Appendix P-II-A. Biosafety Level 1 - Plants (BL1-P)**

#### **Appendix P-II-A-1. Standard Practices (BL1-P)**

##### **Appendix P-II-A-1-a. Greenhouse Access (BL1-P)**

**Appendix P-II-A-1-a-(1).** Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

**Appendix P-II-A-1-a-(2).** Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

##### **Appendix P-II-A-1-b. Records (BL1-P)**

**Appendix P-II-A-1-b-(1).** A record shall be kept of experiments currently in progress in the greenhouse facility.

##### **Appendix P-II-A-1-c. Decontamination and Inactivation (BL1-P)**

**Appendix P-II-A-1-c-(1).** Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

##### **Appendix P-II-A-1-d. Control of Undesired Species and Motile Macroorganisms (BL1-P)**

**Appendix P-II-A-1-d-(1).** A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

**Appendix P-II-A-1-d-(2).** Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

##### **Appendix P-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P)**

**Appendix P-II-A-1-e-(1).** Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

### **Appendix P-II-A-2. Facilities (BL1-P)**

#### **Appendix P-II-A-2-a. Definitions (BL1-P)**

**Appendix P-II-A-2-a-(1).** The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

**Appendix P-II-A-2-a-(2).** The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

#### **Appendix P-II-A-2-b. Greenhouse Design (BL1-P)**

**Appendix P-II-A-2-b-(1).** The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

**Appendix P-II-A-2-b-(2).** Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

#### **Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P)**

##### **Appendix P-II-B-1. Standard Practices (BL2-P)**

###### **Appendix P-II-B-1-a. Greenhouse Access (BL2-P)**

**Appendix P-II-B-1-a-(1).** Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

**Appendix P-II-B-1-a-(2).** Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

###### **Appendix P-II-B-1-b. Records (BL2-P)**

**Appendix P-II-B-1-b-(1).** A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

**Appendix P-II-B-1-b-(2).** A record shall be kept of experiments currently in progress in the greenhouse facility.

**Appendix P-II-B-1-b-(3).** The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/OBA and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent 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). Documentation of any such accident shall be prepared and maintained.

###### **Appendix P-II-B-1-c. Decontamination and Inactivation (BL2-P)**

**Appendix P-II-B-1-c-(1).** Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

**Appendix P-II-B-1-c-(2).** Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

###### **Appendix P-II-B-1-d. Control of Undesired Species and Motile Macroorganisms (BL2-P)**

**Appendix P-II-B-1-d-(1).** A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

**Appendix P-II-B-1-d-(2).** Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

**Appendix P-II-B-1-e. Concurrent Experiments Conducted in the Greenhouse (BL2-P)**

**Appendix P-II-B-1-e-(1).** Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

**Appendix P-II-B-1-f. Signs (BL2-P)**

**Appendix P-II-B-1-f-(1).** A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

**Appendix P-II-B-1-f-(2).** If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

**Appendix P-II-B-1-f-(3).** If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

**Appendix P-II-B-1-g. Transfer of Materials (BL2-P)**

**Appendix P-II-B-1-g-(1).** Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

**Appendix P-II-B-1-h. Greenhouse Practices Manual (BL2-P)**

**Appendix P-II-B-1-h-(1).** A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

**Appendix P-II-B-2. Facilities (BL2-P)**

**Appendix P-II-B-2-a. Definitions (BL2-P)**

**Appendix P-II-B-2-a-(1).** The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

**Appendix P-II-B-2-a-(2).** The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

**Appendix P-II-B-2-b. Greenhouse Design (BL2-P)**

**Appendix P-II-B-2-b-(1).** A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

**Appendix P-II-B-2-b-(2).** Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

**Appendix P-II-B-2-c. Autoclaves (BL2-P)**

**Appendix P-II-B-2-c-(1).** An autoclave shall be available for the treatment of contaminated greenhouse materials.

**Appendix P-II-B-2-d. Supply and Exhaust Air Ventilation Systems (BL2-P)**

**Appendix P-II-B-2-d-(1).** If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

**Appendix P-II-B-2-e. Other (BL2-P)**

**Appendix P-II-B-2-e-(1).** BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

**Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P)****Appendix P-II-C-1. Standard Practices (BL3-P)****Appendix P-II-C-1-a. Greenhouse Access (BL3-P)**

**Appendix P-II-C-1-a-(1).** Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

**Appendix P-II-C-1-a-(2).** Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

**Appendix P-II-C-1-b. Records (BL3-P)**

**Appendix P-II-C-1-b-(1).** A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

**Appendix P-II-C-1-b-(2).** A record shall be kept of experiments currently in progress in the greenhouse facility.

**Appendix P-II-C-1-b-(3).** The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent 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). Documentation of any such accident shall be prepared and maintained.

**Appendix P-II-C-1-c. Decontamination and Inactivation (BL3-P)**

**Appendix P-II-C-1-c-(1).** All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

**Appendix P-II-C-1-d. Control of Undesired Species and Motile Macroorganisms (BL3-P)**

**Appendix P-II-C-1-d-(1).** A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

**Appendix P-II-C-1-d-(2).** Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

**Appendix P-II-C-1-e. Concurrent Experiments Conducted in the Greenhouse (BL3-P)**

**Appendix P-II-C-1-e-(1).** Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.

**Appendix P-II-C-1-f. Signs (BL3-P)**

**Appendix P-II-C-1-f-(1).** A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

**Appendix P-II-C-1-f-(2).** If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

**Appendix P-II-C-1-f-(3).** If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

**Appendix P-II-C-1-g. Transfer of Materials (BL3-P)**

**Appendix P-II-C-1-g-(1).** Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

**Appendix P-II-C-1-h. Greenhouse Practices Manual (BL3-P)**

**Appendix P-II-C-1-h-(1).** A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

**Appendix P-II-C-1-i. Protective Clothing (BL3-P)**

**Appendix P-II-C-1-i-(1).** Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

**Appendix P-II-C-1-i-(2).** Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

**Appendix P-II-C-1-j. Other (BL3-P)**

**Appendix P-II-C-1-j-(1).** Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

**Appendix P-II-C-1-j-(2).** All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

**Appendix P-II-C-2. Facilities (BL3-P)****Appendix P-II-C-2-a. Definitions (BL3-P)**

**Appendix P-II-C-2-a-(1).** The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

**Appendix P-II-C-2-a-(2).** The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

**Appendix P-II-C-2-b. Greenhouse Design (BL3-P)**

**Appendix P-II-C-2-b-(1).** The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

**Appendix P-II-C-2-b-(2).** Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

**Appendix P-II-C-2-b-(3).** The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

**Appendix P-II-C-2-b-(4).** The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

**Appendix P-II-C-2-b-(5).** Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix P-II-C-2-b-(6).** Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix P-II-C-2-b-(7).** The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

**Appendix P-II-C-2-c. Autoclaves (BL3-P)**

**Appendix P-II-C-2-c-(1).** An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

**Appendix P-II-C-2-d. Supply and Exhaust Air Ventilation Systems (BL3-P)**

**Appendix P-II-C-2-d-(1).** An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

**Appendix P-II-C-2-d-(2).** The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

**Appendix P-II-C-2-e. Other (BL3-P)**

**Appendix P-II-C-2-e-(1).** BL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

**Appendix P-II-C-2-e-(2).** Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

**Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P)****Appendix P-II-D-1. Standard Practices (BL4-P)****Appendix P-II-D-1-a. Greenhouse Access (BL4-P)**

**Appendix P-II-D-1-a-(1).** Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

**Appendix P-II-D-1-a-(2).** Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

**Appendix P-II-D-1-a-(3).** Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

**Appendix P-II-D-1-a-(4).** Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

**Appendix P-II-D-1-a-(5).** Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4-P practices and procedures.

**Appendix P-II-D-1-b. Records (BL4-P)**

**Appendix P-II-D-1-b-(1).** A record shall be kept of all experimental materials brought into or removed from the greenhouse.

**Appendix P-II-D-1-b-(2).** A record shall be kept of experiments currently in progress in the greenhouse facility.

**Appendix P-II-D-1-b-(3).** A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

**Appendix P-II-D-1-b-(4).** The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent 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). Documentation of any such accident shall be prepared and maintained.

**Appendix P-II-D-1-c. Decontamination and Inactivation (BL4-P)**

**Appendix P-II-D-1-c-(1).** All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

**Appendix P-II-D-1-c-(2).** Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

**Appendix P-II-D-1-c-(3).** Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

**Appendix P-II-D-1-d. Control of Undesired Species and Motile Macroorganisms (BL4-P)**

**Appendix P-II-D-1-d-(1).** A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

**Appendix P-II-D-1-d-(2).** Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4-P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

**Appendix P-II-D-1-e. Concurrent Experiments Conducted in the Greenhouse (BL4-P)**

**Appendix P-II-D-1-e-(1).** Experiments involving organisms that require a containment level lower than BL4-P may be conducted in the greenhouse concurrently with experiments that require BL4-P containment provided that all work is conducted in accordance with BL4-P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4-P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

**Appendix P-II-D-1-f. Signs (BL4-P)**

**Appendix P-II-D-1-f-(1).** A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

**Appendix P-II-D-1-f-(2).** If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

**Appendix P-II-D-1-f-(3).** If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

**Appendix P-II-D-1-g. Transfer of Materials (BL4-P)**

**Appendix P-II-D-1-g-(1).** Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

**Appendix P-II-D-1-g-(2).** Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.



**Appendix P-II-D-1-h. Greenhouse Practices Manual (BL4-P)**

**Appendix P-II-D-1-h-(1).** A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

**Appendix P-II-D-1-i. Protective Clothing (BL4-P)**

**Appendix P-II-D-1-i-(1).** Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

**Appendix P-II-D-1-i-(2).** Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

**Appendix P-II-D-1-i-(3).** All laboratory clothing shall be autoclaved before laundering.

**Appendix P-II-D-2. Facilities (BL4-P)****Appendix P-II-D-2-a. Greenhouse Design (BL4-P)**

**Appendix P-II-D-2-a-(1).** The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.

**Appendix P-II-D-2-a-(2).** Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

**Appendix P-II-D-2-a-(3).** Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

**Appendix P-II-D-2-a-(4).** Access doors to the greenhouse shall be self-closing and locking.

**Appendix P-II-D-2-a-(5).** The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

**Appendix P-II-D-2-a-(6).** The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix P-II-D-2-a-(7).** Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix P-II-D-2-a-(8).** A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

**Appendix P-II-D-2-b. Autoclaves (BL4-P)**

**Appendix P-II-D-2-b-(1).** A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

### **Appendix P-II-D-2-c. Supply and Exhaust Air Ventilation Systems (BL4-P)**

**Appendix P-II-D-2-c-(1).** An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

**Appendix P-II-D-2-c-(2).** Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

### **Appendix P-II-D-2-d. Other (BL4-P)**

**Appendix P-II-D-2-d-(1).** Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

**Appendix P-II-D-2-d-(2).** A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

**Appendix P-II-D-2-d-(3).** Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

**Appendix P-II-D-2-d-(4).** If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

### **Appendix P-III. Biological Containment Practices**

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

#### **Appendix P-III-A. Biological Containment Practices (Plants)**

**Appendix P-III-A-1.** Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

**Appendix P-III-B. Biological Containment Practices (Microorganisms)**

**Appendix P-III-B-1.** Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

**Appendix P-III-C. Biological Containment Practices (Macroorganisms)**

**Appendix P-III-C-1.** Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

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**APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING ANIMALS**

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede [Appendix G \(Physical Containment\)](#) when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see [Appendix Q-III-D, Footnotes and References for Appendix Q](#)). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

**Appendix Q-I. General Considerations**

**Appendix Q-I-A. Containment Levels**

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in [Section III, Experiments Covered by the NIH Guidelines](#). For the purpose of animal research, four levels of containment are established. These are referred to as BL1-Animals (N), BL2-N, BL3-N, and BL4-N and are described in the following appendices of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

**Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N)**

**Appendix Q-I-B-1.** When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

**Appendix Q-I-B-2.** A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

**Appendix Q-II. Physical and Biological Containment Levels****Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N)****Appendix Q-II-A-1. Standard Practices (BL1-N)****Appendix Q-II-A-1.a. Animal Facility Access (BL1-N)**

**Appendix Q-II-A-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-A-1-a-(2).** Access to the containment area shall be limited or restricted when experimental animals are being held.

**Appendix Q-II-A-1-a-(3).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-A-1.b. Other (BL1-N)**

**Appendix Q-II-A-1-b-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-A-1-b-(2)** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-A-1-b-(3).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-A-2. Animal Facilities (BL1-N)**

**Appendix Q-II-A-2.a.** Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

**Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N)** (See [Appendix Q-III-A](#), *Footnotes and References for Appendix Q*)

**Appendix Q-II-B-1. Standard Practices (BL2-N)****Appendix Q-II-B-1.a. Animal Facility Access (BL2-N)**

**Appendix Q-II-B-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-B-1-a-(2).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-B-1-a-(3).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-B-1-a-(4).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

**Appendix Q-II-B-1-a-(5).** Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

**Appendix Q-II-B-1-b. Decontamination and Inactivation (BL2-N)**

**Appendix Q-II-B-1-b-(1).** Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

**Appendix Q-II-B-1-b-(2).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-B-1-c. Signs (BL2-N)**

**Appendix Q-II-B-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-B-1-d. Protective Clothing (BL2-N)**

**Appendix Q-II-B-1-d-(1).** Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

**Appendix Q-II-B-1-d-(2).** Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

**Appendix Q-II-B-1-e. Records (BL2-N)**

**Appendix Q-II-B-1-e-(1).** Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent 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). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-B-1-e-(2).** When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

**Appendix Q-II-B-1-f. Transfer of Materials (BL2-N)**

**Appendix Q-II-B-1-f-(1).** Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

**Appendix Q-II-B-1-g. Other (BL2-N)**

**Appendix Q-II-B-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-B-1-g-(2).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-B-1-g-(3).** Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

**Appendix Q-II-B-1-g-(4).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-B-1-g-(5).** Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-B-1-g-(6).** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-B-1-g-(7).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-B-1-g-(8).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-B-2. Animal Facilities (BL2-N)**

**Appendix Q-II-B-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

**Appendix Q-II-B-2-b.** Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix Q-II-B-2-c.** The animal containment area shall be designed so that it can be easily cleaned.

**Appendix Q-II-B-2-d.** Windows that open shall be fitted with fly screens.

**Appendix Q-II-B-2-e.** An autoclave shall be available for decontamination of laboratory wastes.

**Appendix Q-II-B-2-f.** If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

**Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N)** (See [Appendix Q-III-B](#), *Footnotes and References for Appendix Q*)

**Appendix Q-II-C-1. Standard Practices (BL3-N)**

**Appendix Q-II-C-1-a. Animal Facility Access (BL3-N)**

**Appendix Q-II-C-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-C-1-a-(2).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-C-1-a-(3).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-C-1-a-(4).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

**Appendix Q-II-C-1-a-(5).** Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

**Appendix Q-II-C-1-b. Decontamination and Inactivation (BL3-N)**

**Appendix Q-II-C-1-b-(1).** The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

**Appendix Q-II-C-1-b-(2).** All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

**Appendix Q-II-C-1-b-(3).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-C-1-b-(4).** Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

**Appendix Q-II-C-1-b-(5).** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

**Appendix Q-II-C-1-c. Signs (BL3-N)**

**Appendix Q-II-C-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-C-1-d. Protective Clothing (BL3-N)**

**Appendix Q-II-C-1-d-(1).** Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3-N area and wearing of personal clothing.

**Appendix Q-II-C-1-d-(2).** Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

**Appendix Q-II-C-1-d-(3).** Appropriate respiratory protection shall be worn in rooms containing experimental animals.

#### **Appendix Q-II-C-1-e. Records (BL3-N)**

**Appendix Q-II-C-1-e-(1).** Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

**Appendix Q-II-C-1-e-(2).** Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent 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). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-C-1-e-(3).** When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

#### **Appendix Q-II-C-1-f. Transfer of Materials (BL3-N)**

**Appendix Q-II-C-1-f-(1).** Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

**Appendix Q-II-C-1-f-(2).** Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

#### **Appendix Q-II-C-1-g. Other (BL3-N)**

**Appendix Q-II-C-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-C-1-g-(2).** Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

**Appendix Q-II-C-1-g-(3).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-C-1-g-(4).** Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.



**Appendix Q-II-C-1-g-(5).** Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3-N practices.

**Appendix Q-II-C-1-g-(6).** Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

**Appendix Q-II-C-1-g-(7).** All procedures shall be performed carefully to minimize the creation of aerosols.

**Appendix Q-II-C-1-g-(8).** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-C-1-g-(9).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-C-1-g-(10).** All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

**Appendix Q-II-C-1-g-(11).** Personnel shall be required to shower before exiting the BL3-N area and wearing personal clothing.

**Appendix Q-II-C-1-g-(12).** Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

**Appendix Q-II-C-1-g-(13).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-C-1-g-(14).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

#### **Appendix Q-II-C-2. Animal Facilities (BL3-N)**

**Appendix Q-II-C-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

**Appendix Q-II-C-2-b.** The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix Q-II-C-2-c.** Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

**Appendix Q-II-C-2-d.** An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-C-2-e.** If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

**Appendix Q-II-C-2-f.** Access doors to the containment area shall be self-closing.

**Appendix Q-II-C-2-g.** The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

**Appendix Q-II-C-2-h.** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

**Appendix Q-II-C-2-i.** An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

**Appendix Q-II-C-2-j.** If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

**Appendix Q-II-C-2-k.** Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix Q-II-C-2-l.** In lieu of open housing in the special animal room, animals held in a BL3-N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

**Appendix Q-II-C-2-m.** Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

**Appendix Q-II-C-2-n.** Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

**Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N)** (See [Appendix Q-III-C](#), *Footnotes and References for Appendix Q*)

**Appendix Q-II-D-1. Standard Practices (BL4-N)**

**Appendix Q-II-D-1-a. Animal Facility Access (BL4-N)**

**Appendix Q-II-D-1-a-(1).** Individuals under 16 years of age shall not be permitted to enter the animal area.

**Appendix Q-II-D-1-a-(2).** The containment area shall be locked.

**Appendix Q-II-D-1-a-(3).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-D-1-a-(4).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-D-1-a-(5).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

**Appendix Q-II-D-1-a-(6).** Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

**Appendix Q-II-D-1-a-(7).** Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

**Appendix Q-II-D-1-a-(8).** Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

**Appendix Q-II-D-1-b. Decontamination and Inactivation (BL4-N)**

**Appendix Q-II-D-1-b-(1).** All contaminated liquid or solid wastes shall be decontaminated before disposal.

**Appendix Q-II-D-1-b-(2).** The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

**Appendix Q-II-D-1-b-(3).** All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

**Appendix Q-II-D-1-b-(4).** No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix Q-II-D-1-b-(5).** When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

**Appendix Q-II-D-1-b-(6).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-D-1-b-(7).** Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

**Appendix Q-II-D-1-b-(8).** An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-D-1-b-(9).** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

**Appendix Q-II-D-1-c. Signs (BL4-N)**

**Appendix Q-II-D-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-D-1-d. Protective Clothing (BL4-N)**

**Appendix Q-II-D-1-d-(1).** Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4-N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

**Appendix Q-II-D-1-d-(2).** A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

**Appendix Q-II-D-1-d-(3).** Appropriate respiratory protection shall be worn in rooms containing experimental animals.

**Appendix Q-II-D-1-e. Records (BL4-N)**

**Appendix Q-II-D-1-e-(1).** Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

**Appendix Q-II-D-1-e-(2).** A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable). Reports to the NIH/OBA shall be sent 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). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-D-1-e-(3).** When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

**Appendix Q-II-D-1-e-(4).** A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

**Appendix Q-II-D-1-f. Transfer of Materials (BL4-N)**

**Appendix Q-II-D-1-f-(1).** No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix Q-II-D-1-f-(2).** Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4-N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4-N animal facility to one with a lower containment classification.

**Appendix Q-II-D-1-f-(3).** Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

#### **Appendix Q-II-D-1-g. Other (BL4-N)**

**Appendix Q-II-D-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-D-1-g-(2).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-D-1-g-(3).** Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-D-1-g-(4).** Experiments involving other organisms that require containment levels lower than BL4-N may be conducted in the same area concurrently with experiments requiring BL4-N containment provided that they are conducted in accordance with BL4-N practices.

**Appendix Q-II-D-1-g-(5).** Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

**Appendix Q-II-D-1-g-(6).** All procedures shall be performed carefully to minimize the creation of aerosols.

**Appendix Q-II-D-1-g-(7).** A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

**Appendix Q-II-D-1-g-(8).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-D-1-g-(9).** The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

**Appendix Q-II-D-1-g-(10).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall

not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-D-1-g-(11).** An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

**Appendix Q-II-D-1-g-(12).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-D-1-g-(13).** Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

#### **Appendix Q-II-D-2. Animal Facilities (BL4-N)**

**Appendix Q-II-D-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

**Appendix Q-II-D-2-b.** The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix Q-II-D-2-c.** Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

**Appendix Q-II-D-2-d.** An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-D-2-e.** Access doors to the containment area shall be self-closing.

**Appendix Q-II-D-2-f.** All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

**Appendix Q-II-D-2-g.** The BL4-N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

**Appendix Q-II-D-2-h.** A necropsy room shall be provided within the BL4-N containment area.

**Appendix Q-II-D-2-i.** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

**Appendix Q-II-D-2-j.** A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

**Appendix Q-II-D-2-k.** Exhaust air from BL4-N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4-N containment area.

**Appendix Q-II-D-2-l.** All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least  $1 \times 10^{-3}$  micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80-120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

**Appendix Q-II-D-2-m.** If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally  $1 \times 10^8$  organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilis*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

**Appendix Q-II-D-2-n.** All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

**Appendix Q-II-D-2-o.** Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

**Appendix Q-II-D-2-p.** Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

**Appendix Q-II-D-2-q.** The supply water distribution system shall be fitted with a back-flow preventer or break tank.

**Appendix Q-II-D-2-r.** All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

**Appendix Q-II-D-2-s.** Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

### **Appendix Q-III. Footnotes and References for Appendix Q**

**Appendix Q-III-A.** If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

**Appendix Q-III-B.** Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3-N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

**Appendix Q-III-C.** Risk Group 4 and restricted microorganisms (see [Appendix B](#), *Classification of Human Etiologic Agents on the Basis of Hazard*, and [Sections V-G and V-L](#), *Footnotes and References of Sections I through IV*) pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import animal or plant pathogens, special approval must be obtained from [U.S. Department of Agriculture, Animal and Plant Health Inspection Service \(APHIS\)](#), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, MD 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

**Appendix Q-III-D.** Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in [Appendix Q](#), may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in [Appendices G](#) and [P](#).

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Institutional Biosafety Committee (IBC)

**Arizona State University**

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Tempe, Arizona 85287-6111

(480) 965-4387 FAX: (480) 965-7772

### Biosafety Disclosure Review

**Disclosure Number:** 12-452

**Form:** 112AB rDNA & Infectious Agents

**Project Title:** Rewiring the Histone Code with Synthetic Biology

**Principal Investigator:** Karmella Haynes

**Containment Level:** BSL 2

**Approval Date:** 02/16/2012

**Expiration Date:** 02/15/2015

The Biosafety disclosure review was considered by the IBC Chair or Committee and the following decisions were made:

- The original disclosure was APPROVED as presented.
- The revised disclosure was APPROVED as presented.
- The disclosure was APPROVED with STIPULATIONS as noted below.
- The Committee requests CLARIFICATIONS or CHANGES in the disclosure as described in the attached memorandum. The disclosure will be reconsidered when these issues are clarified and the revised disclosure is submitted.
- The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
- The Committee requests you to contact \_\_\_\_\_ to discuss this proposal.
- NIH Recombinant DNA Experiment Classification Category for this disclosure:**  
Section III-F-6 Appendix C-I and C-II.

**REQUIRED STIPULATIONS:** None

**TRAINING:** Current.


**NOTE:** Although protocol approval is for three years, please note that an annual review form EHS 112AR must be submitted annually to record any changes or modifications to the protocol. The Office of Research Integrity and Assurance will send you an annual reminder.

Signature:

C. Miller for D. Murphy Date: 2/17/2012  
IBC Chair or Designee

For IBC Use Only: IBC # 12-452

BSL Level 2

 <p><b>Arizona State University</b> Institutional Biosafety Committee</p>	<p><b>RECOMBINANT DNA &amp; INFECTIOUS AGENT DISCLOSURE FORM EHS 112</b></p>
<p><b>NOTE:</b> Forms need to be completed and submitted to the IBC at least two weeks in advance of the meeting date. Meeting dates are posted on the IBC website at <a href="http://researchintegrity.asu.edu/biosafety/meetings">http://researchintegrity.asu.edu/biosafety/meetings</a>. Submit disclosure forms to <a href="mailto:research.integrity@asu.edu">research.integrity@asu.edu</a>, or by fax to 965-7772, or mail code 6111. Late submissions will be reviewed the following month.</p>	

New Submission     
  Renewal – Previous IBC#     
  Sponsored Project

<b>PRINCIPAL INVESTIGATOR:</b>	Karmella Haynes	Phone No:	480-965-4636
Department:	SBHSE	Fax No:	480-727-7624
Building, Office Room No., Mail Code	ECG 346, 9709	Email:	karmella.haynes@asu.edu
Campus where research will be performed: <input checked="" type="checkbox"/> Tempe <input type="checkbox"/> West <input type="checkbox"/> Poly <input type="checkbox"/> Downtown			
<b>CO-INVESTIGATOR:</b>	n/a	Phone No:	
Department:		Fax No:	
Building, Office Room No., Mail Code		Email:	
<b>PROJECT TITLE(S):</b>	Rewiring the histone code with synthetic biology		
Funding Agency:	n/a	ASU Proposal or Award #: New faculty start-up	
Dates of Project:	From: 9/2011	To:	9/2012

The University's Institutional Biosafety Committee (IBC) is comprised of both active researchers and lay persons. Regardless of backgrounds, each member has one vote, and it is therefore particularly important that the language of the application be understood by all. This applies to all sections of the application, but it is especially important that the goals and justifications of the proposed research be spelled out in the clearest possible terms.

**NOTE:** Upon approval, this disclosure may become a public record, so please do not disclose proprietary information.

Provide a brief (300 words or less) synopsis in laymen terms of proposed research. Artificial proteins will be designed in ways that chemical drugs cannot, in order to change the expression of natural genes in living cells while minimizing off-target toxic effects. Fragments of DNA will be assembled using molecular cloning techniques, to build artificial genes that express synthetic proteins in human cell lines (in culture). The synthetic proteins are designed to control the expression of natural genes in the cells. We aim to determine the specificity and effectiveness of this new class of synthetic proteins, and to control natural genes could lead to the inhibition of cancer growth or guided differentiation of stem cells for tissue regeneration.

The DNA fragments used in our studies come from microbial, viral, and human proteins that have no known toxic or pathogenic properties. DNA assembly will be aided by maintaining circularized partially-built intermediates (plasmids) in a non-pathogenic bacteria (E. coli K12). The assembled DNA (recombinant DNA) will be introduced into cells via a lipid-based method ("transfection"), not via viral infection. We will use several cell lines (HEK293 human kidney, U2OS human osteosarcoma) that carry a stably integrated recombinant gene. Our plasmids will carry resistance to ampicillin, neomycin, puromycin, and zeomycin in order to select for bacteria and human cells that carry the gene.

1. What do you believe is the required Biosafety Containment Level (BSL 1, BSL 2, BSL 3)? BSL 2  
[http://www.asu.edu/uagc/EHS/documents/infectious\\_agents.pdf](http://www.asu.edu/uagc/EHS/documents/infectious_agents.pdf)

2. Experiments involve the use of (check all that apply):  
Please list specific cell lines and if purchasing from an outside source (e.g. ATCC), please include the product number.

- A.  Human Subjects IRB Protocol Number:
- B.  Whole Animals, species: IACUC Protocol Number:
- C.  Transgenic Animals, type:  
Does the research involve the creation of new transgenic animals?  Yes  No
- D.  Parasites, list:
- E.  Whole Plant, list:
- F.  Bacteria, list: DH5-alpha
- G.  Fungi, list:
- H.  Viruses, list:
- I.  Recombinant DNA (please complete Recombinant DNA or Gene Transfer Section and the Recombinant DNA form found at <http://researchintegrity.asu.edu/biosafety/forms>)
- J.  Agents known or suspected to be infectious to humans, animals, or plants
- K.  Human or NHP blood, bloodborne pathogens, blood products, tissue, or cells (including cell cultures), list: immortalized cell lines HEK293 and U2OS
- L.  Select Agents:

For a list of Select Agents and Toxins visit the CDC website:  
<http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html>.

If you are working with a Select Agent, please complete form RSK112C found at  
<http://researchintegrity.asu.edu/biosafety/forms>.

M.  Select Toxins:

Regulated Select Toxins include:

- Abrin
- Botulinum neurotoxins
- Clostridium perfringens epsilon toxin
- Conotoxin
- Diacetoxyscirpenol (DAS)
- Ricin
- Saxitoxin
- Shiga-like ribosome inactivating proteins
- Shigatoxin
- Staphylococcal enterotoxins
- T-2 toxin
- Tetrodotoxin

3. List all rooms where experiment(s) will be conducted. (Include building and room #) SCOB290 is only being used for BSL1 work

- a. Lab ISTB1 230A; SCOB 290 Campus:  Tempe  West  Poly  Downtown working with bacteria in sealed tubes.
- b. Animal Housing Campus:  Tempe  West  Poly  Downtown ISTB1 257 will also be used for experiments.
- c. Are there any other rooms where the materials will be stored when not in use?  Yes  No

List all rooms: Campus  Tempe  West  Poly  Downtown

4. EH&S Lab Registration Date(s) for lab(s) listed in question #3 (See Lab Door Signage): not yet done

5. Does this study involve a transfer of biological materials to or from ASU?  Yes  No

- a. If yes, will an MTA be used to transfer the material?  Yes  No
6. Describe safety procedures specific to this research. Please attach copies of any applicable SOP's.
- a. Identify potential exposure hazards during sample preparation and experimental manipulations (e.g. aerosol generation when transferring, mixing or centrifuging, use of sharps, excretion by animals, etc.)  
**Cells in culture, non-pathogenic bacteria** Cultured mammalian cells suspended in liquid medium, non-pathogenic bacteria suspended in liquid medium and grown on agar, sharps contaminated with cells/bacteria (pipette tips, broken glass & plastic)
- b. What safety procedures will be employed to minimize risk and prevent release of infectious agents (e.g. protective clothing, use of biological safety cabinet, sharps disposal procedures, waste disposal procedures, etc.)?  
**Collection and disinfection of all supernatant culture media; disposal of sharps into biohazard sharps containers; biohazard disposal and autoclaving of materials that have come into contact with living/unfixed cells; PPE (full-length lab coats, close-toed shoes); food and drink prohibited in lab area (including desks) , Eye protection and gloves will also be worn as part of PPE.**
- c. List accidental spill and exposure procedures.
- **Spill: Surround the spill with paper towels, cover the spill with paper towels, treat the towels with 10% bleach, discard in biohazard waste container, clean area with 10% bleach, discard towels in biohazard bin**
  - **Exposure: wash skin completely with anti-bacterial soap and water**
  - **Broken skin exposure: wash area, report to EH&S**
- d. Describe detailed disposal procedures.
- **Disinfect liquids with a final concentration of bleach at 10%; request pick-up**
  - **Collect solid waste into lined biohazard bin; autoclave; request pick-up**

Identify all personnel conducting the experiments (including students and staff). Specify degree, applicable training and experience including duration (e.g. 2 years), and project responsibilities. Biosafety training is required for BSL2 and BSL3 disclosures. Biosafety training is **NOT** the same as Laboratory Safety training.

NAME	ASURITE NAME	DEGREE	TRAINING/EXPERIENCE	Biosafety Training Date Completed (mm/yy) Req'd for BSL2 & BSL3	PROJECT RESPONSIBILITIES
PI: Karmella Haynes	khaynes5	Ph.D.	>10 years	11/11 11/18/11	P.I.
Caroline Hom	cmhom	none	none	11/11 12/19/11	Human cell culture, cloning
Brady Laughlin	bslaugh1	none	none	11/11 11/15/11	Molecular cloning (bacteria)
Derek Sanchez	djsanche	B.S.	none	pending 11/23/12	Molecular cloning (bacteria)
Behzad Damadzadeh	bdamadza	M.S.	4 years 2/13/12	pending <del>11/23/12</del>	Human cell culture, cloning

**Recombinant DNA or Gene Transfer (if this section is not applicable, sign and submit application)**

1. What genes will you be working with? List them below. Please explain abbreviations.

Gene	Species of Origin
Fluorescent proteins (mCherry, YFP, AmCyan)	synthetic
VP16 core transcription factor, Thymidine kinase core promoter	Herpes simplex virus
P65, SP1, CBX8	Human
Polycomb	Drosophila (fruit fly)
CBX2	Danio rerio (zebra fish)
CMV and CMVTetO2 promoters	Cytomegalovirus

Gal4 DNA binding peptide, Gal4 recognition sites, Kozak sequence	Yeast
Bovine growth hormone polyadenylation signal (BGH polyA)	Cow
polyadenylation signal, nuclear localization signal	Simian virus 40

2. Will the DNA clones contain genes for the biosynthesis of toxic molecules lethal for vertebrates:  
 YES  NO If yes, please provide LD50 information:
3. What is the maximum total volume of culture being used at any one time? 1 liter
4. Are you using plasmids?  Yes  No  
 a. To what organisms will the genes be transferred?  
 viruses (list below)  
 bacteria (list below) (If *E. coli*, indicate if it is *E. coli* K-12 or other)  
 other (list below)

Name of virus or bacteria receiving DNA
<i>E. coli</i> K-12 (DH5-alpha)
Human cell culture HEK293, U2OS

5. If cloning DNA into virus:  
 a. Describe how plasmids/DNA will be used to create the virus.  
 n/a  
 b. To what host(s) will the viruses be transferred?  
 c. Do experiments involve formation of recombinant DNA molecules containing  $\geq 2/3$  of the genome of any eukaryotic virus?  Yes  No  
 d. Do experiments involve the use of infectious human, animal, insect, or plant viruses?  Yes  No  
 e. Do experiments involve the use of defective animal or plant viruses in the presence of helper virus?  
 Yes  No - if yes, **attach** your procedures to determine the relative proportions of helper virus and defective virus in potentially mixed virus stock.  
 f. Are the genes likely to make the virus more pathogenic?  Yes  No (if yes, please explain)
6. If cloning DNA into bacteria:  
 Are the genes likely to make the bacteria more pathogenic?  Yes  No (if yes, please explain)

Attach a detailed map of all vectors and inserts to be used. Please indicate any regions that increase the safety of this construct. Provide copies of key references that describe the construction of the vector(s) to be used.

By signing below you are agreeing that all work on this project will be conducted using biosafety practices described in the CDC/NIH Publication entitled *Biosafety in Medical and Biomedical Laboratories (BMBL)*. Additional stipulations required by the Institutional Biosafety Committee on behalf of Arizona State University will also be followed.

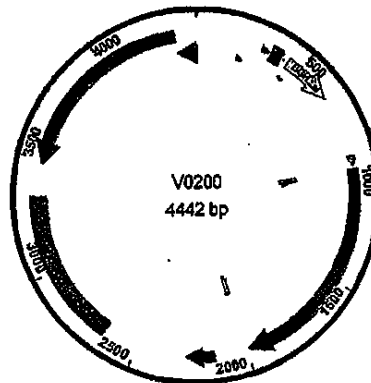
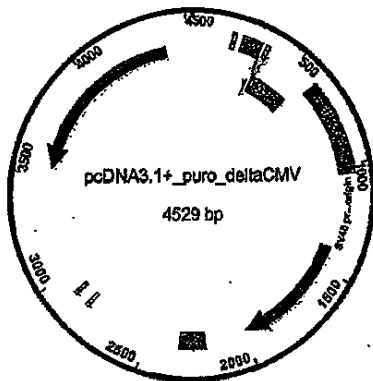
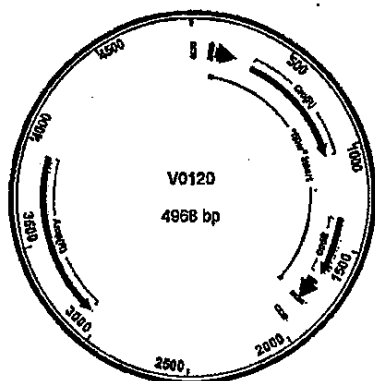
Principal Investigator's Signature submitted via email Date: 2/16/12

Send completed form to: IBC, Office of Research Integrity and Assurance, Mail Code 6111 or FAX 965-7772

FOR IBC COMMITTEE USE ONLY		
Application is	Signature	Date
<input checked="" type="checkbox"/> Approved by Committee/IBC Chair		2/16/12

Maps of all vectors and inserts to be used. Annotated sequences for all DNA used in our lab are archived at the MIT Registry of Standard Biological Parts.

Vectors:



**V0120**  
 Safety: ccdB causes bacterial death when transferred to most strains.  
 Reference: [http://partsregistry.org/Part:BBa\\_I176127](http://partsregistry.org/Part:BBa_I176127)

**pcDNA3.1+ puro delta CMV**  
 Reference: [http://partsregistry.org/Part:BBa\\_I176122](http://partsregistry.org/Part:BBa_I176122)  
 Modified versions carry different markers...  
 Neomycin (G418) resistance: [http://partsregistry.org/Part:BBa\\_I176128](http://partsregistry.org/Part:BBa_I176128)  
 Hygromycin resistance: [http://partsregistry.org/Part:BBa\\_I176123](http://partsregistry.org/Part:BBa_I176123)  
 Zeocin resistance: [http://partsregistry.org/Part:BBa\\_I176124](http://partsregistry.org/Part:BBa_I176124)

**V0200**  
 Contains FRT site for stable integration into chromosomal DNA of cells that have been modified to carry a target FRT site  
 Reference: [http://partsregistry.org/Part:BBa\\_I176121](http://partsregistry.org/Part:BBa_I176121)

Most commonly used synthetic gene inserts carried by the vectors above:



Over-expressed synthetic transcription factor. Variants carry different domains with similar functions. See [http://partsregistry.org/Part:BBa\\_I176062](http://partsregistry.org/Part:BBa_I176062) for one example.

1. Promoters (cytomegalovirus): constitutively active CMV, or CMVTetO2 which is active only in the presence of doxycycline
2. Kozak (yeast): protein translation start site
3. Binding domains: Polycomb (Drosophila), CBX8 (human), CBX2 (zebra fish), Gal4 DNA binding peptide (yeast)
4. Fluorescent tag: mCherry red (synthetic)
5. Transcription activator: VP16 tetramer (HSV), P65 (human), SP1 (human)
6. Targeting domain: SV40 nuclear localization signal (simian virus 40)
7. Epitope tag: myc (human)
8. Polyadenylation signal: bovine growth hormone (cow) or simian virus 40



Synthetic reporter gene. Variants carry different domains with similar functions. See [http://partsregistry.org/Part:BBa\\_I176104](http://partsregistry.org/Part:BBa_I176104) for one example.

1. Binding sites: Gal4 (yeast)
2. Promoter (HSV): weak thymidine kinase core promoter
3. Kozak (yeast): protein translation start site
4. Fluorescent tag: mCherry red (synthetic), YFP yellow (synthetic), AmCyan blue (synthetic)
5. Targeting domain: SV40 nuclear localization signal (simian virus 40)
6. Polyadenylation signal: bovine growth hormone (cow) or simian virus 40



# Recombinant DNA Experiment Classifications



(Check any that apply-see bottom of page for Risk Group definitions)

- Section III-F-1:** Experiments that are not in organisms or viruses.
- Section III-F-2:** Experiments that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, although one or more of the segments may be a synthetic equivalent.
- Section III-F-3:** Experiments 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.
- Section III-F-4:** Experiments that consist entirely of DNA from an 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-5:** 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 is prepared and periodically revised by the NIH Director and can be found at [http://oba.od.nih.gov/oba/rac/Guidelines/NIH\\_Guidelines.pdf](http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf)
- Section III-F-6:** Those exemptions as determined by the NIH Director to not present a significant risk to health or the environment are listed in the appendices below. Please check all categories that apply:
  - Appendix C-I:** Recombinant DNA In Tissue Culture: Molecules Containing <1/2 of any Eukaryotic Viral Genome.
  - Appendix C-II:** *Escherichia coli* K-12 Host-Vector Systems.
  - Appendix C-III:** *Saccharomyces* Host-Vector Systems.
  - Appendix C-IV:** *Kluyveromyces* Host-Vector Systems.
  - Appendix C-V:** *Bacillus Subtilis* or *Bacillus Licheniformis* Host-Vector Systems.
  - Appendix C-VI:** Extrachromosomal Elements of Gram Positive Organisms.
  - Appendix C-VII:** The Purchase or Transfer of Transgenic Rodents: BSL 1 only.
  - Appendix C-VIII:** Transgenic Rodents Generated by Breeding, BSL 1 only.
- Section III-E:** Experiments that are not included in Sections III-A, III-B, III-C, III-D, and III-F; and experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic eukaryotes fall under Section III-E and may be conducted at **BSL-1 containment**.
- 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 (**BSL 1 only**).
- Section III-E-2:** Experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA modified organisms associated with whole plants (**BSL 1 only**).
- Section III-E-3:** Experiments involving transgenic rodents, modified by the stable introduction of genetic material. Note: This section applies to BLS 1 only; all others are classified under Section III-D-4.
- Section III-D-1:** Experiments using Risk Group 2, Risk Group 3, or restricted agents as host-vector systems.  
Select Risk Group:  Risk Group 2 (RG2)  Risk Group 3 (RG3)
- Section III-D-2:** Experiments in which DNA from Risk Group 2, Risk Group 3, or restricted agents is cloned into non-pathogenic prokaryotic or lower eukaryotic host-vector systems.  
Select Risk Group:  Risk Group 2 (RG2)  Risk Group 3 (RG3)  Risk Group 4 (RG4)
- Section III-D-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.  
Select Risk Group:  Risk Group 2 (RG2)  Risk Group 3 (RG3)
- Section III-D-4:** Experiments involving whole animals (e.g., non-human vertebrate or invertebrate organism, including arthropods).  
Select Section that applies:  Section III-D-4-a: Risk Group 1 Organisms  Section III-D-4-b: Risk Group 2 or 3 Organisms
- Section III-D-5:** Experiments involving whole plants or insects; experiments to genetically engineer plants by recombinant DNA methods, to use such plants for experimental purposes (e.g. response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA (cannot be done at BSL 1).
- Section III-D-6:** Experiments involving more than 10 liters of culture.

Please note: This section requires NIH pre-approval. Please contact the IBC for assistance.

- Section III-A-1:** The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally. (Requires RAC review and NIH Director pre-approval)
- Section III-B-1:** Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight. (Requires NIH pre-approval)
- Section III-C-1:** Experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants. (Requires NIH pre-approval)

## Risk Group Definitions

**Risk Group 1 (RG1):** Agents that are not associated with disease in healthy adult humans

**Risk Group 2 (RG2):** Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.

**Risk Group 3 (RG3):** Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.

**Risk Group 4 (RG4):** Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available.

PI Signature

Karmella Haynes

Date

11/18/11

Disclosure #

12-452

Rev. Date 9/29/2011



**BIOLOGICAL SAFETY LEVEL TWO (BSL2) INSPECTION CHECKLIST**

**Introduction:** ASU's research and teaching laboratories that operate at Biological Safety Level Two (BSL2) are required to have annual inspections by EH&S biosafety professionals. This checklist will be used during the annual inspections. It is the intent of EH&S to provide laboratories with this checklist to help identify safety and regulatory deficiencies and address them before their annual inspections occurs. Please use this checklist to perform your own self-inspection of your laboratory. If you check "N" or No to any of these questions, please correct this finding. In doing so, your laboratory will comply with regulations and make a safer working environment for anyone who works or enters your laboratory. If you have any questions, please contact [Biosafety@asu.edu](mailto:Biosafety@asu.edu).

**Date:** \_\_\_\_\_

**Lab Locations:** \_\_\_\_\_

**PI:** \_\_\_\_\_

**Inspector:** \_\_\_\_\_

**Lab Representative:** \_\_\_\_\_

Standard Practices		Question
Y	N	NA
A1		Do personnel wash their hands after they handle biohazardous materials, after removing gloves, and before leaving the lab?
A2		Is mouth pipetting prohibited and are mechanical pipetting devices used?
A3		Is eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption prohibited in the lab?
A4		Are all procedures performed carefully in a manner to minimize the creation of splashes or aerosols?
A5		Are work surfaces decontaminated with an effective disinfectant on completion of work or at the end of the day, and especially after overt spills or splashes of biohazardous materials?
A6		Are all syringes/needles/sharps disposed of in rigid, puncture-resistant, leak-proof containers?
A7		Are all wastes that are contaminated with biohazardous materials autoclaved or decontaminated with an effective disinfectant before they are scheduled for pick-up?
A8		Is there an SOP for proper autoclave use posted next to the autoclave?
<b>Special Practices</b>		
B1		Does the PI advised personnel who work in the lab about the potential hazards and specific entry requirements (e.g., immunization)?
B2		Has the PI developed lab-specific biosafety procedures and incorporated them into either a Biosafety Manual or Standard Operating Procedures?
B3		Are lab personnel required to read and fill out Lab-Specific Biosafety Checklist?





**BIOLOGICAL SAFETY LEVEL TWO (BSL2) INSPECTION CHECKLIST**

B4		Are needle-locking syringes or safety hypodermic needles used when appropriate?
B5		Do lab personnel understand that used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated?
B6		Do lab personnel use mechanical means, such as a brush and dustpan, tongs, or forceps to clean up broken glassware?
B7		Are cultures, tissues and other biohazardous materials placed in a container with a cover that prevents leakage during collection, handling, processing, storage, or transport?
B8		Do lab personnel notify the PI immediately if there are spills and accidents that result in exposures to biohazardous materials?
B9		Is medical follow-up obtained if appropriate?
B10		Are all laboratory personnel and particularly women of childbearing age provided with information regarding immune competence and conditions that may predispose them to infection and encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance?
<b>Safety Equipment</b>		
C1		If there is a biological safety cabinet in the lab, has it been certified within the past year?
C2		Is the biological safety cabinet free of equipment or supplies that can block the air grills and disrupt proper airflow?
C3		Is a biological safety cabinet used for all procedures with a potential for creating biohazardous aerosols or splashes? These may include: grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of biohazardous materials (especially those internal pressures may differ from ambient pressures), inoculating animals intra-nasally, harvesting infected tissues from animals or embryonated eggs.
C4		Is a biological safety cabinet used when high concentrations or large volumes of biohazardous materials are handled?
C5		When lab personnel use vacuum lines with biohazardous materials, are they protected with High Efficiency Particulate Air (HEPA) filters?
C6		Is equipment for use or storage of biohazardous materials (i.e. refrigerator, freezers) labeled with a biohazard symbol?
C7		When lab personnel centrifuge biohazardous materials, do they use sealed rotor heads or centrifuge safety cups and open the rotors or safety cups only in a biological safety cabinet?
<b>Personal Protective Equipment (PPE)</b>		
D1		Do personnel wear lab coats whenever they are in the lab and remove them before leaving the lab?
D2		Are personnel prohibited from taking their lab coats home for laundering?
D3		Do personnel wear gloves to prevent contact with biohazardous materials?
D4		Do personnel remove gloves before touching "clean" surfaces (keyboards, telephones, elevators, etc.) and before leaving the lab?
D5		When biohazardous materials must be manipulated outside a biological safety cabinet, do personnel use eye and face protection?
D6		Are alternatives to powdered latex gloves available for personnel with latex sensitivity?



**BIOLOGICAL SAFETY LEVEL TWO (BSL2) INSPECTION CHECKLIST**

Lab Facilities	
E1	Is a BIOHAZARD sign posted on the lab entrance door which includes the biosafety level, any required immunizations, emergency contact numbers, and any personal protective equipment that must be worn in the lab?
E2	Does the lab have a current registration placard on the entrance door?
E3	Does the lab contain a sink for handwashing?
E4	Are bench tops impervious to water and resistant to moderate heat and the chemicals used to decontaminate the work surfaces and equipment?
E5	Are biological safety cabinets located away from doors, windows that can be opened, heavily traveled lab areas, and other potentially disruptive equipment?
E6	Are carpets and rugs kept out of the lab?
E7	Are chairs and other furniture used in the lab covered with a non-fabric material that can be easily decontaminated?
Bloodborne Pathogens	
X1	Have lab personnel been offered and received appropriate immunizations for the agents potentially present in the lab (e.g., hepatitis B)? Or declined in writing?
X2	Do lab personnel have access to the <i>ASU Bloodborne Pathogens Exposure Control Plan</i> ?
X3	Have all lab personnel with the potential for exposure to bloodborne pathogens or other potentially infectious materials completed ASU's Bloodborne Pathogens Training program?
Recombinant DNA	
Y1	Do lab personnel have access to the <i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i> ?
Y2	Has the PI's recombinant DNA research been reviewed and approved by the Institutional Biosafety Committee?
Y3	Do lab personnel have access to copies of SOPs for recombinant DNA materials?
Y4	Is the PI familiar with which section of the <i>NIH Guidelines</i> their research falls under?
Y5	Are lab personnel familiar with the emergency response procedures for spills or exposures involving rDNA material?
Survey Section	
1	Are you working with or generating any toxins of biological origin? Which toxin and how many mg?
2	Have you noticed any pest control issues in your lab or office?



## STANDARD OPERATING PROCEDURE (SOP) FOR BIOLOGICAL SAFETY CABINET OPERATION

Biological Safety Cabinet Make/Model: \_\_\_\_\_

Person Responsible and Phone #: \_\_\_\_\_

Located in Building/Room: \_\_\_\_\_

### I. PURPOSE

The purpose of this guideline is to detail the safe operation of biological safety cabinets (BSC) in ASU laboratories and to ensure adequate containment of biological materials. The most common type of BSC at ASU is a Class II. Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and handling of infectious organisms.

### II. RESPONSIBILITIES

#### A. Principal Investigator

The Principal Investigator (PI) is responsible for ensuring personnel under their supervision are trained on the safe and proper use of the BSC and that correct procedures are followed.

#### B. Designated Person

The PI may designate a member of the lab to oversee the correct operation and maintenance of the BSC. This person shall:

- Periodically inspect the BSC to ensure its operational performance.
- Coordinate yearly BSC certification by the certified vendor. The dates of the most recent certification must be posted on the front of the BSC.
- Make any necessary arrangements for repairs and annual certification of the BSC.
- Report to PI unsafe practices by BSC users.
- Label the BSC when it is not operating correctly.

#### C. BSC Users

- BSC users must receive proper training from their PI on the safe and proper use of the BSC prior to use.
- BSC users must follow correct procedures.
- BSC users shall report any injuries, accidents or spills to their PI.
- BSCs users shall report any defects or malfunctions of the BSC to their PI or the designated person.

### III. DEFINITIONS

**Biological Safety Cabinet:** A piece of equipment designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating substances containing infectious agents, such as viruses, bacteria and primary tissue cultures.

**High Efficiency Particulate Air (HEPA) Filter:** Filter that traps 99.97% of particles of 0.3  $\mu\text{m}$  in diameter and 99.99% of particles of greater or smaller size, thus capturing all infectious agents and ensuring only microbe-free air is exhausted from the cabinet or directed to the work surface.

### IV. PROCEDURES

#### A. Prior to Operation

- Read the Material Safety Data Sheets (MSDS) for materials being used in a BSC. Note any precautions regarding the use of the chemical or microorganisms in the BSC.
- Lift the sash to the recommended height.
- Turn on the BSC fan 10 minutes before beginning work to allow adequate air filtration.
- Check the air intake and exhaust grilles for obstructions, and check the pressure gauge reading indicating HEPA filter load. If you do not know the acceptable reading for the cabinet, ask the certifier or consult your manufacturer's manual.
- If the cabinet is equipped with an alarm, test the alarm and switch it to the "ON" position.
- Confirm inward airflow by holding a piece of tissue at the middle of the edge of the viewing panel and ensuring it is drawn in.
- Decontaminate the cabinet surface and surface-decontaminate all materials to be placed inside the BSC (refer to the Cleaning and Disinfection section).
- Bulky items, such as waste containers and suction collection flasks, should be placed to one side of the interior of the cabinet.
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may impede proper airflow and the level of protection provided. Consider using wire racks or shelving to increase airflow around front and rear grills.
- Wear eye protection, a lab coat, closed-toe shoes and disposable gloves to protect yourself as well as your samples from contamination.
- Respirators may be required for some procedures. The nature of the Personal Protective Equipment (PPE) worn may vary according to the findings obtained from the biological risk assessment.

#### B. Operation

- Active work should flow from clean to contaminated areas across the work surface.
- Arms should be moved in and out slowly, perpendicular to the front opening to minimize disruption of the air curtain and laminar flow.
- Work as far to the back as possible, but within comfortable reach.
- Always use mechanical pipetting aids. Mouth pipetting is not allowed.
- Heat sources such as Bunsen burners are strictly prohibited inside the BSCs as they significantly disrupt the laminar flow of air.
- To sterilize bacteriological loops, micro-burners or electric "furnaces" may be used.
- Do not work in a BSC while a warning light or alarm is signaling.

- Locate liquid waste traps inside cabinet and use an in-line HEPA filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a durable plastic tray or box) to prevent breakage.

### C. Spills

**In the event of a spill within a BSC, follow these steps:**

1. Alert the other laboratory employees.
2. Leave the cabinet turned on.
3. While wearing gloves, spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
4. Report the spill to the laboratory's PI, who will report the spill to the RO if a select agent or toxin is involved.
5. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
6. Autoclave all clean-up materials before disposal in the biohazard waste container.
7. Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

### D. Cleaning and Disinfection

- When work is completed, all equipment and supplies from the BSC should be decontaminated and removed from the cabinet.
- The interior surfaces should also be wiped with an appropriate disinfectant that would kill any microorganisms that could be found in the cabinet. Corrosive chemicals such as bleach should be avoided, but if used, should be followed with a wipe down of sterile water or 70% ethanol.
- Allow the cabinet to run for 10 minutes.

### E. Ultraviolet (UV) Lights

- Ultraviolet (UV) lamps are not recommended in BSCs nor are they necessary.
- If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light.
- The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted.
- UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer.
- If the cabinet has a sliding sash, close the sash when operating the UV lamp.

### F. Other Considerations

- All repairs to the BSC must be performed by a qualified technician. Any malfunction of the BSC must be reported and repaired before the BSC is used again.
- The BSC should be positioned in an isolated corner to minimize disruption of the air intake arising from traffic around the biosafety cabinet or drafts from doors, windows and/or air conditioning.
- The BSC must be certified by an NSF/ANSI Standard 49 qualified technician.

- The BSC must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination may only be performed by a certified contractor.
- Contact [Biosafety@asu.edu](mailto:Biosafety@asu.edu) for questions.

## V. REFERENCES

[EH&S Biosafety](http://cfo.asu.edu/ehs-biosafety) (<http://cfo.asu.edu/ehs-biosafety>)

[ASU Biosafety Manual](http://www.asu.edu/uagc/EHS/documents/biosafetymanual.pdf), (<http://www.asu.edu/uagc/EHS/documents/biosafetymanual.pdf>)

[NSF 49 Standard](http://www.nsf.org/business/biosafety_cabinetry/index.asp?program=BiosafetyCab), ([http://www.nsf.org/business/biosafety\\_cabinetry/index.asp?program=BiosafetyCab](http://www.nsf.org/business/biosafety_cabinetry/index.asp?program=BiosafetyCab))

[CDC, BMBL 5<sup>th</sup> Ed.](http://www.cdc.gov/biosafety/publications/bmb15/index.htm), (<http://www.cdc.gov/biosafety/publications/bmb15/index.htm>)



## STANDARD OPERATING PROCEDURE (SOP) FOR AUTOCLAVE OPERATION

Autoclave Make/Model: \_\_\_\_\_

Person Responsible and Phone #: \_\_\_\_\_

Located in Building/Room: \_\_\_\_\_

### I. PURPOSE

The purpose of this document is to provide standard operating procedures for the use of autoclaves. Autoclaving is a process used to destroy microorganisms and decontaminate biohazardous waste and microbiological equipment used at Biosafety Level 1, 2, 3 and 4.

### II. RISK MANAGEMENT

#### A. Potential Risks

Autoclaves use high pressure and high temperature steam for sterilization. The potential safety risks for the operators include:

- ✓ **Heat burns** from hot materials and autoclave chamber walls and door.
- ✓ **Steam burns** from residual steam coming out from autoclave and materials on completion of cycle.
- ✓ **Hot fluid scalds** from boiling liquids and spillage in autoclave and during transport of superheated materials.
- ✓ **Hand and arm** injuries when closing the door.
- ✓ **Body** injury if there is an explosion.

#### B. Health and Safety

To insure the health and safety of personnel using the autoclave, it is important for each department to maintain autoclaves and to train personnel in their proper use.

- The name of the person responsible for the autoclave shall be posted near the autoclave. This SOP should be posted on the outside to the autoclave.
- It is the supervisor's responsibility to ensure employees are trained before operating any autoclave unit.
- Procedural and instructional documents provided by the manufacturer must be followed.
- Personal protective clothing and equipment must be worn when loading and unloading the autoclave.

- Autoclaves must be inspected at least annually. Inspection services may be managed by your manufacturer's preventative maintenance contract. A basic visual inspection should be performed monthly by the person responsible for the autoclave. The inspection, service and repair records should be available upon request.
- Spore strips may be used to validate autoclave effectiveness.

### C. Personal Protective Equipment (PPE)

Equipment to protect against scalds and burns include:

- Heat-insulating gloves that provide complete coverage of hands and forearms.
- Lab coat.
- Eye protection.
- Closed-toe footwear.

## III. OPERATOR INSTRUCTIONS

### A. Training

All personnel who use autoclaves must have successfully completed a training session from their supervisor on the safe operating procedures. This requirement applies to both new and experienced personnel.

### B. Material Preparation

- Ensure that the material is safe for autoclaving:
  - Samples containing solvents or substances that may emit toxic fumes should not be autoclaved.
  - Do not autoclave bleach!
- Glassware must be inspected for cracks prior to autoclaving.
- Prepare and package material suitably:
  - Loose dry materials must be wrapped or bagged in steam-penetrating paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing effectiveness of the process.
  - Loosen all lids to prevent pressure buildup. All containers must be covered by a loosened lid or steam-penetrating bung.
  - Containers of liquid must not exceed two-thirds (2/3) full, with lids loosened.
  - Glassware must be heat-resistant borosilicate.
  - Plastics must be heat-resistant, i.e., polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items.



- Discarded sharps must be in a designated 'Sharps' container.
- All items must be tagged with autoclave tape.
- Place items in secondary containers to secure and contain spills:
  - Items should be placed in a stainless steel pan or other autoclavable container for their stability and ease of handling.
  - Place containers of liquid, bags of agar plates, or other materials that may boil over or leak, into a secondary pan in the autoclave.
  - The pan must be large enough to contain a total spill of the contents.
  - Bags must not be tightly sealed as steam cannot penetrate.
- Biohazardous waste must be processed according to ASU guidelines described online at <http://cfo.asu.edu/ehs-biowaste-compliance-guideline>.

### C. Loading Autoclave

- Wear lab coat, eye protection, heat-insulating gloves, and closed-toe shoes.
- Place material in autoclave. Do not mix incompatible materials.
- Do not overload; leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air.
- Close and latch door firmly.

### D. Operating Autoclave

- Close and lock door.
- Choose appropriate cycle (i.e., gravity, liquid, or dry cycle) for the material. Consult the autoclave manual for assistance in choosing a cycle. The manuals for operation of the autoclave should be located near the autoclave.
- Set appropriate time and temperature if you are using a customized cycle.
- Start your cycle and fill out the autoclave user log with your contact information. A completed cycle usually takes between 1-1.5 hours, depending on type of cycle.
- Do not attempt to open the door while autoclave is operating.
- If problems with your autoclave are perceived, abort cycle and report it to your PI immediately.

### E. Unloading Autoclave

- Wear heat-insulating gloves, eye protection, lab coat, and closed-toe shoes.
- Ensure that the cycle has completed and both temperature and pressure have returned to a safe range.
- Wearing PPE, stand back from the door as a precaution and carefully open door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.

- Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.
- Wearing heat-insulated gloves remove items from the autoclave and place them in an area which clearly indicates the items are 'hot' until the items cool to room temperature.
- Shut autoclave door.

**Note:** Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.

#### **F. Autoclave Use Log**

- Entries must be placed in the log book each time the autoclave is used. These records are used for maintenance/service schedules and reporting of incidents, accidents and/or faults.
- Entries should include: operator's name, phone number, date, time and duration.
- The log book must be kept adjacent to the autoclave.
- An Autoclave Use Log example is provided in this document.

#### **G. Maintenance and Repair**

- No person shall operate the autoclave unless the autoclave is in good repair.
- Only qualified professionals are permitted to make repairs.
- Report possible malfunctions to \_\_\_\_\_.
- Repairs are performed by your service contract or any other contractor you choose to hire.

### **IV. CONTINGENCY PLANS**

#### **A. Equipment Malfunction**

- If the autoclave does not operate exactly as expected, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.
- Record the problem in the autoclave log book.
- Contact \_\_\_\_\_ or your supervisor to report the problem.
- Only qualified professionals are permitted to make repairs.

#### **B. Incident Response**

- All incidents, including a spill or release of biohazardous materials or recombinant DNA, must be reported to your supervisor and EH&S at 965-1823.
- If any injury occurs seek first aid or, if necessary, seek medical assistance at ASU Health Services or by dialing 911.
- If clothing is soaked in hot water/steam, remove clothing and place the injury in cool water.

- Place a notice on the autoclave indicating that it is not to be used until the cause of the incident is determined, procedures enacted to prevent future incidents, and the autoclave is deemed safe for operation.

### C. Spill Clean-up

- Spills may occur from a boil-over or breakage of containers.
- No operation of the autoclave is allowed until the spill is cleaned up.
- The operator is responsible for clean-up of spills. Contain the spilled material using paper towels. Use your laboratory's spill kit if necessary. Wait until the autoclave and materials have cooled to room temperature before attempting clean-up.
- Review the MSDS, if appropriate, to determine appropriate PPE, spill cleanup and disposal protocols that are necessary.
- Dispose of the waste following the protocol appropriate for the material (i.e., red biohazard bag). If materials have been intermingled, follow the clean-up and disposal protocol for the most hazardous component of the mixture.
- Cracked glassware must be disposed of properly.
- Record the spill and clean-up procedure in the autoclave log book.

## V. REFERENCES

EH&S Biosafety: <http://cfo.asu.edu/ehs-biosafety>

Waste Pickup Request: <http://cfo.asu.edu/ehs-environmentalaffairs>

ASU Biosafety Manual: <http://www.asu.edu/uagc/EHS/documents/biosafetymanual.pdf>

CDC, BMBL 5<sup>th</sup> Edition: <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

### AUTOCLAVE USE LOG

Print Name (No initials)	Contact Number	Date	Time	Cycle	Comments

## Hazardous Materials Incident

### Only Trained Authorized Personnel Are Permitted to Respond to Hazardous Materials Incidents

A **hazardous materials incident** is considered a spill of any of the following:

Chemicals (liquid and solids)  
Hazardous waste or  
Oils (diesel and gasoline).

#### For a Major Spill or Leak

- Activate the nearest fire alarm.
- Immediately evacuate the area, closing doors behind you.
- **Call 911**. The ASU Police Department will contact Environmental Health & Safety (EH&S).
- DO NOT attempt to clean up the spill yourself.
- Provide clean-up/response personnel with appropriate Materials Safety Data Sheets (MSDS) and other pertinent information.

#### Nights and weekends call the ASU Police by campus

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West 602.543.3456 | Downtown 602.496.3456

#### For a Minor Spill or Leak

**Contact Environmental Health & Safety (EH&S) @ 480.965.1823 M-F 8am-5pm.**

**Nights and weekends call the ASU Police Department @ 480.965.3456.**

- Alert people in the immediate area of the spill.
- Wear proper personal protective equipment (PPE) and attempt to contain the spill.
- Avoid breathing vapors from the spill. Confine spill to a small area.
- DO NOT ALLOW ANYTHING TO DISCHARGE INTO DRAINS!
- Bag cleanup material and label it with Hazardous Waste Label.
- Contact EH&S at 480.965.3899 for removal.

#### Chemical Spill on Body

- Flood exposed area with running water from faucet or safety shower for at least 15 minutes.
- Remove contaminated clothing at once. Put in a plastic bag and seal. Avoid contact with eyes.
- Make sure chemical has not accumulated in shoes or under jewelry.
- If no visible burn, check MSDS to determine if delayed effects may be expected.

#### Obtain medical attention at the Campus Health by campus:

**Tempe 480.965.3349 | Poly 480.727.1500 | West 602.543.8019 | Downtown 602.496.0721 M-F 9am-4:30pm** or other appropriate resources - even for minor chemical burns.

Report incident to supervisor.

**Supervisor responsible to notify ASU EH&S @ 480.965.1823 M-F 8am-5pm**

**Nights and weekends call the ASU Police by campus:**

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West

602.543.3456 | Downtown 602.496.3456

ASU Environmental Health & Safety has personnel trained in the proper clean-up and containment of minor hazardous spills or releases.

**Call 480.965.1823 M-F 8am-5pm**

**Nights and weekends call the ASU Police by campus:**

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West  
602.543.3456 | Downtown 602.496.3456

Employees must be trained in the safe use of chemicals and chemical waste management prior to working in a laboratory or cleaning up minor spills. A minor spill is one that trained employees are capable of containing safely without assistance from EH&S and /or emergency personnel. All other spills are considered major.

#### **Additional Site Instructions**

- Location of the Spill Response Kit:
- Location of nearest safety shower:
- Location of nearest eyewash:
- Location of telephone:
- Location of Material Safety Data Sheets (MSDS)

## Biological Spills

### Notes and Precautions

A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment is: concentrated disinfectant (household bleach or organism specific disinfectant), paper towels, rubber gloves, autoclave bags, sharps container, and forceps to pick up broken glass.

For any biological spill, first notify others in the laboratory so they will not unknowingly spread the contamination. For spills of a large volume, or spills of organisms transmitted by inhalation when aerosolized such as **BioSafety Level 3** (BSL3) agents, call the Principal Investigator (PI) and EH&S at 480.965.1823 Mon-Fri 8am-5pm.

### Nights and weekends call the ASU Police by campus

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West 602.543.3456 | Downtown 602.496.3456

If someone is injured call 911 for assistance; notify emergency personnel if you are contaminated.

### Spill of BSL2 material or Human Blood

- Wear gloves, safety glasses and lab coat. If splashing is likely, wear goggles, or a face shield.
- Use forceps to pick up broken glass and discard into SHARPS container.
- Cover spilled material with paper towels.
- Carefully pour diluted disinfectant onto paper towels in sufficient quantity to ensure effective microbial inactivation.
- Allow a 20-minute contact period.
- Pick up paper towels and dispose in biohazard waste container.
- Re-wipe spill area with disinfectant diluted to working strength.
- Place all contaminated materials, including Personal Protective Equipment (PPE), into biohazard waste container and autoclave.
- Wash hands with soap and water.

### Spill of BSL3 material

- Stop work. Alert others to evacuate laboratory immediately. Avoid inhaling airborne material.
- Close doors to affected area; post a sign "Do not enter."
- Remove contaminated clothing. Turn exposed area inward and place in a biohazard bag (preferably in the ante-room). Decontaminate the bottom of your shoes.
- Wash hands and other contaminated skin with soap and water.
- Notify PI and EH&S at 480.965.1823 Mon-Fri, 8am-5pm.
- **Nights and weekends call the ASU Police by campus**  
Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West 602.543.3456 | Downtown 602.496.3456 and indicate if you need assistance.
- Call 911 if there is an injury (when calling 911 from a cell phone, ask for ASU police except Downtown campus).
- Do not re-enter laboratory for at least 30 minutes to allow aerosols to disperse.
- Assemble all clean-up materials.
- Re-enter lab and cover spill with paper towels or disposable pads. Pour diluted disinfectant onto paper towels.
- Leave the room for 30 minutes to allow the disinfectant to inactivate the material.

- Pick up any broken glass with forceps and dispose in SHARPS container.
- Pick up paper towels and wipe the area again with an appropriate disinfectant.
- Place all contaminated materials, including disposable PPE, in a biohazard bag and autoclave.
- Wash hands thoroughly with soap and water.
- Make arrangements through your PI to receive a post-exposure medical evaluation.

#### Spill on Body

- Remove contaminated clothing; put it in a plastic bag and seal it.
- Vigorously wash exposed area with soap and water for 1 minute
- **Obtain medical attention at the Campus Health by campus:  
Tempe 480.965.3349 | Poly 480.727.1500 | West 602.543.8019 | Downtown 602.496.0721  
M-F 9am-4:30pm** or other appropriate resources.
- Report incident to supervisor. Supervisor is responsible for notifying ASU EH&S at 480.965.1823 Mon-Fri, 8am-5pm.
- **Nights and weekends call the ASU Police by campus**  
Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West 602.543.3456|  
Downtown 602.496.3456 and indicate if you need assistance.

#### Biosafety Risk Groups

Risk Group 1 / BSL 1	Agents that are not associated disease in healthy adult humans
Risk Group 2 / BSL 2	Agents that are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available
Risk Group 3 / BSL 3	Agents that are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available
Risk Group 4 / BSL 4	Agents that are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available. None at ASU.

#### Additional Site Instructions

- Location of the Spill Response Kit:
- Location of nearest safety shower:
- Location of nearest eyewash:
- Location of telephone:
- Location of Material Safety Data Sheets (MSDS):
- Telephone number for PI:



## Evacuation

In advance of an emergency, contact your department head for additional emergency preparedness related documentation/information.

Always be prepared. Learn all exits and routes to help determine the nearest exit to your location and the best route to follow if evacuation is necessary.

If time permits during evacuation, and it is safe to do so, secure your workplace and take personal items.

In most emergencies, complete evacuation of the campus is not necessary. If, however, there is a major hazardous materials release, flood, or other MAJOR incident, it may be necessary to relocate all University personnel to a safer location.

### Evacuation from a Building:

- Walk, do not run.
- Do not use elevators.
- If safe to do so, assist people with special needs as indicated by that person, or direct to the nearest stairwell, and contact the ASU Police Department at 911 for assistance with location.
- Gather outside at a designated staging area. There your supervisor will take roll and account for all personnel.
- If you cannot return to your building, wait for instructions from the ASU Police Department or other organization in charge.

### “In-Place” Evacuations

In some instances, it is safer to evacuate “in-place” than it is to leave a building, e.g., smoke or fire is immediately outside your room, live electrical wires bar access to the exit, individuals with mobility disabilities are above or below ground floors.

- If the hazard is fire or smoke, see “**FIRE**” section of this guide.
- If the hazard causes elevators to become inoperative (fire alarm sounds).
- If safe to do so, go to the nearest stairwell and tell someone who is evacuating to notify emergency personnel of your location and that you are unable to evacuate the building.
- Call 911 and tell them your name, your location, that you are unable to evacuate, and why you are unable to evacuate the building. Follow directions of the 911 operator.

### On-Campus Staging Areas

In the event of a building or area evacuation, you will be notified where staging areas will be located. Food, water, first-aid, shelter, and information will be available at designated staging areas

## Medical Emergency

All university personnel are encouraged to learn first-aid and CPR. For more information call ASU Environmental Health & Safety (EH&S) at 480.965.1823 Mon-Fri, 8am-5pm

### Call 911 From Any Campus Phone

- Before proceeding with the following emergency responses on all medical emergencies!
- The guidelines below are for ADULT rescue
- For individuals under the age of 8, follow the dispatcher's instructions

### Start Rescue Breaths - If Victim is Not Breathing

- Tilt the victim's head back, lift the chin, pinch the nose shut
- Give 2 short 1 second breaths. (Breathe into victim until chest gently rises)
- Check for circulation. (Normal breathing, coughing, movement)
- If there are signs of circulation, but victim is still NOT BREATHING, give one slow breath every 5 seconds (10 to 12 breaths per minute)
- Re-check signs of circulation and breathing every minute. Continue rescue breathing as long as victim is not breathing or until medical assistance arrives
- If there are no signs of circulation and victim is still not breathing, initiate Cardio Pulmonary Resuscitation (CPR)

### Start CPR - If Victim is Not Breathing and No Signs of Circulation

- Find the notch where lower ribs meet the breastbone. Place the heel of your hand on the breastbone. Place your other hand on top of the first.
- Position shoulders over hands. Compress chest 30 times (1-1/2 to 2 inches) using a smooth, even rhythm.
- Give 2 short 1 second breaths.
- Do five (5) more sets of (30) compressions and (2) breaths (approximately 100 compressions per minute).
- Check for signs of circulation.
- If there are signs of circulation, but victim is still NOT BREATHING, give one slow breath every 5 seconds (10 to 12 times per minute)
- If there are no signs of circulation and victim is not breathing, continue CPR.

### Use Automated External Defibrillator (AED) If Available

- If the building is equipped with AEDs, retrieve the closest unit.
- If lay responder(s) are trained in the use of an AED, proceed with using unit.
- If lay responder(s) are not trained in the use of an AED, follow the dispatcher's instructions.
- Do not use the AED on a patient who is lying in water or on any electrically conductive surface such as wet grass.
- Do not operate the AED in a potentially explosive environment.
- **NOTE:** The use of an AED is necessary to revive a victim of cardiac arrest. The unit is automated and will only shock when necessary.
- If an AED was used, contact ASU EH&S at 480.965.1823 Mon-Fri, 8am-5pm. Nights and weekends call the ASU Police Department.

### ASU Police by Campus

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West 602.543.3456 | Downtown 602.496.3456

### **Choking (Abdominal Thrusts)**

- Call 911.
- Get behind victim; wrap your arms around the person's waist, just above their navel.
- Clasp your hands together in a double fist. PRESS IN AND UP IN QUICK THRUSTS.
- Be careful not to exert pressure against the victim's rib cage with forearms.
- Repeat procedure until choking stops.
- Report incident to supervisor. Supervisor is responsible for notifying ASU EHS at 480.965.1823 Mon-Fri, 8am-5pm

### **Nights and weekends call the ASU Police by campus**

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West  
602.543.3456 | Downtown 602.496.3456

### **Additional Site Instructions**

- Location of the nearest AED:
- Location of telephone:

## Personal Injury

In all "serious cases" call 911 for emergency treatment

### Heart Related Illnesses

- Get victim to a cool place.
- Loosen tight clothing.
- Apply cool, wet cloths to the skin.
- Fan the victim.
- If victim is conscious, give cool (not cold) water to drink.
- Obtain medical attention at the Campus Health by campus:  
Tempe 480.965.3349| Poly 480.727.1500 | West 602.543.8019| Downtown 602.496.0721 M-F 9am-4:30pm or other appropriate resources.
- Call 911 for an ambulance, if victim refuses water, vomits, or loses consciousness.
- Report incident to supervisor. Supervisor is responsible for notifying ASU EH&S @ 480.965.1823 Monday – Friday 8:00 a.m. – 5:00 p.m. Nights and weekends call the ASU Police by campus:  
Tempe 480.965.3456  
Poly 480.727.3456 or 480.727.3456 after hours  
West 602.543.3456|  
Downtown 602.496.3456

### Minor Cuts & Punctures

- Vigorously wash injury with soap and water for several minutes.
- Obtain medical attention at the Campus Health by campus:  
Tempe 480.965.3349| Poly 480.727.1500 | West 602.543.8019| Downtown 602.496.0721 M-F 9am-4:30pm or other appropriate resources - to check tetanus status.
- If **IMPALED** by a foreign object – **DO NOT REMOVE THE OBJECT**. Seek medical attention immediately.
- Report incident to supervisor. Supervisor is responsible for notifying ASU EH&S @ **480.965.1823** Monday – Friday 8:00 a.m. – 5:00 p.m. Nights and weekends call the ASU Police by campus: Tempe **480.965.3456**|Poly **480.727.3456** or **480.727.3456** after hours| West **602.543.3456**| Downtown **602.496.3456**.

### Material Splashed in Eye

- Immediately rinse eye and inner surface of eyelid with water continuously for 15 minutes.
- Forcibly hold eye open to ensure effective wash behind eyelids.
- Obtain medical attention at the Campus Health by campus: Tempe **480.965.3349**| Poly **480.727.1500** | West **602.543.8019**| Downtown **602.496.0721** M-F 9am-4:30pm or other appropriate resources

Report incident to supervisor. Supervisor is responsible for notifying ASU EH&S at **480.965.1823** Mon-Fri, 8am-5pm. Nights and weekends call the ASU Police by campus:

Tempe **480.965.3456** | Poly **480.727.3456** or **480.727.3456** after hours | West **602.543.3456** | Downtown **602.496.3456**

### Site Instructions:

- Location of First Aid Kit:

- Location of nearest safety shower:
- Location of nearest eyewash:
- Location of telephone:

## Fire

**All employees are required to attend fire safety/fire prevention training. Register for a class**

SEE “**EVACUATION**” SECTION OF THIS GUIDE FOR MORE INFORMATION.

### **If You Discover a Fire or See Smoke**

- If building alarm is not sounding, manually activate the alarm by using a fire alarm pull station located near an exit.
- Immediately exit the building.
- Call 911 from the nearest safe phone or activate an emergency blue light call box.
- NOTE: Dialing 911 from a cell phone may not go directly to the ASU Police Department. Give the dispatch operator your ASU campus and address.

### **If Building Fire Alarm is Activated or Someone Informs You of a Fire**

- Walk to the nearest exit. **DO NOT USE THE ELEVATORS**
- If able, assist people with special needs.
- Notify emergency personnel if you know or suspect someone is trapped or still inside the building.
- Gather outside at a designated area away from the building and do not attempt to re-enter the building until authorized to do so by the emergency responders.

### **If Caught in Smoke**

- Drop to hands and knees and crawl towards the nearest exit.
- Stay low as smoke will rise to ceiling level.
- Hold your breath as much as possible; breathe through your nose and use a filter such as a shirt or towel.

### **If Trapped in a Room**

- Close as many doors as possible between you and the fire.
- Wet and place a cloth material around or under the door to help prevent smoke from entering the room.
- If room has an outside window, be prepared to signal someone outside.

### **Clothing on Fire: [Stop, Drop, Roll]**

- Roll person around on floor to smother flame.
- Only drench with water if safety shower is immediately available.
- Obtain medical attention. Call 911.

Report incident to supervisor. Supervisor is responsible for notifying ASU EH&S at 480.965.1823 Mon-Fri, 8am-5pm

### **Nights and weekends call the ASU Police by campus**

Tempe 480.965.3456 | Poly 480.727.3456 or 480.727.3456 after hours | West 602.543.3456 | Downtown 602.496.3456

### **Using a Fire Extinguisher**

Only use a fire extinguisher if the fire is very small and you have been trained to do it safely. If you can't put out the fire, leave immediately and make sure the building alarm is activated and emergency personnel notified. **TOTAL AND IMMEDIATE EVACUATION IS THE SAFEST.**

**Additional Site Instructions**

- Location of designated assembly area:
- Location of exit stairwells:
- Location of fire extinguisher:
- Locations of emergency pull stations:
- Location of nearest safety shower and eyewash:
- Location of telephone:

- A hazards analysis with hazard management techniques (engineering type controls)
- Expected methods of chemical and biological waste disposal

This information demonstrates ASU’s efforts to eliminate hazards in the laboratory and our commitment to minimize hazardous wastes generated by the university. If upon review, the information is insufficient or procedures are deemed too hazardous, EH&S may require further analysis or safety measures.

Products of the research and the chemicals remaining after research is complete are also subject to review by EH&S. The researcher must contact EH&S to determine if the chemical waste produced by the experiment is disposable.

There are many rules and regulations controlling disposal of wastes, and this creates many different costs and management problems. The analysis includes a justification for producing these wastes. Analyzing these materials before experimentation begins can prevent many problems not envisioned at the completion of the experiment.

**Personal Protective Equipment (PPE)**

The PI, with the assistance of the CO or EH&S should perform a hazard evaluation to determine which PPE are required for each laboratory task. PPE requirements are covered during laboratory safety training and must be followed by all employees and visitors to the laboratory. Any deviations from the PPE requirements covered during laboratory safety training are to be documented with a PPE hazard assessment analysis. Prior to each use, verify PPE is in good working condition.

Standard operating procedures should include methods used to implement control measures for reducing employee’s exposures to hazardous chemicals and materials.

Engineering controls,

Administrative Controls

Personal Protective Equipment (hygiene practices are possible control measures)

PPE Hazard Analysis forms may be downloaded from [EH&S](#). Copy of a form can be found in [Appendix F](#) of this document. Additional information can be obtained by reviewing the OSHA Personal Protective Equipment Standard, [29 CFR § 1910.132](#).

**PPE Criteria**

\*ANSI criteria for protective equipment are as follows: This form was taken from Arizona State Risk Management training handout for OSHA 1910.132

Protective Equipment	Purchased after July 5, 1994		Purchased before July 5, 1994	
Eye & Face Protection	ANSI Z87.1	1989	ANSI Z87.1	1968
Head Protection	ANSI Z89.1	1986	ANSI Z89.1	1969
Foot Protection	ANSI Z41	1991	ANSI Z41.1	1967



PPE is required to be used at all times while in the laboratory and includes, but may not necessarily be limited to:

- Safety goggles or face shield
- Laboratory coat or other suitable clothing which covers the arms and legs completely (long sleeved shirt and long pants)
- Closed-toe shoes

PPE required to be used at all times when handling potentially hazardous chemicals, reproductive toxins, carcinogens, and sensitizers in the laboratory includes, but is not limited to:

- Appropriate gloves <https://cfo.asu.edu/ehs-gloves>.
- [Approved respirators](#) in the absence of adequate ventilation, e.g., glove boxes or fume hoods
- [Hearing protection devices](#) may be required if noise hazards are present in the laboratory.

### **Lab Coats – Proper Use and Care**

Principal Investigators should ensure that their staff and students wear laboratory coats in the laboratory. If a risk assessment specifies that protective clothing is necessary, laboratory workers must be supplied with, and wear a lab coat when working with any potentially hazardous materials identified as requiring the lab coat. However, EH&S recommends that lab coats be worn at all times in laboratories where potentially hazardous materials are present. Lab coats must not be taken home or to public laundries to be cleaned. Laboratory coats used in the laboratory must not be taken out of the laboratory into any meeting room, break area or dining facility.

Lab coats used for flammable materials should be made of 100% cotton or FR rated materials. EH&S recommends the use of flame retardant lab coats for any use or potential exposure to pyrophoric liquids or gases. Many lab coats are made from cotton/polyester fabric blends. These materials should not be used with open flames or pyrophoric materials.

If a hazardous material is splashed on a lab coat, the wearer is to remove it to prevent the splash from coming into contact with wearer's street clothes or skin. If a splash occurs, remove the lab coat immediately and determine if street clothes or skin have been contaminated. If street clothes are contaminated with corrosives, or materials toxic by skin absorption, remove the street clothes and immediately wash the affected area(s) in an emergency safety shower. Report the incident to your supervisor. Follow your lab's emergency procedures.

### **Wearing and storage:**

- Wear the right size – One size DOES NOT fit all, laboratory coats should fit wearer properly.
- Lab coats must be buttoned to protect street clothing and body from splattering of reagents or blood and body fluids.
- Sleeves must extend beyond the wrist to protect the arm – Do not roll up the sleeves for ventilation or comfort.

- Do not access pant pockets via side slits available in some laboratory coats – Hazardous or infectious materials may be transferred by dirty hands.
- Remove laboratory coat before leaving laboratory.
- If a lab coat is needed in another location, you may wear it in the hallway leading to the other location, but not in an elevator or stairwell. If you need to transport your lab coat to another location, verify it is not contaminated and fold it and carry it to the next location it will be needed, preferably in a clean container such as a bag or back pack.
- Hang up in-use laboratory coats on hooks in a designated area.
- Store clean laboratory coats separately from soiled ones or those in-use.

**Before sending to contract laundry:**

- Check and have laboratory coats cleaned and washed at regular intervals or when they are dirty whichever is earlier.
- The laboratory coat should be either free from obvious contamination or autoclaved before sending to the laundry.
- If the laboratory coat cannot be safely decontaminated or satisfactorily made safe to be handled by the laundry, it should be disposed of accordingly.
- If the laboratory coat is heavily stained with chemical or biological splash, it should be discarded immediately via the appropriate waste route.
- Replace torn or old laboratory coats.

If the laboratory coat is contaminated, the guidelines for pre-laundry treatment are:

- If the chemical spilt on the laboratory coat is safe to be disposed to the sewage (e.g. most acids and alkalis), rinse the laboratory coat with plenty of water.
- If a solvent is spilt on the laboratory coat, it should be completely evaporated in the fume hood first.
- The laboratory coat may have to be disposed of via chemical waste if the contaminant is a particularly hazardous chemical. Consult EH&S for guidance.
- If it is a biological contaminant (e.g. blood, urine, sputum, etc) or microorganisms, the laboratory coat should be autoclaved first. Do not autoclave lab coats that are contaminated with chemicals.
- Do not send radioactive isotope-contaminated laboratory coats to the laundry. Contact Radiation Safety for guidance.
- Place laboratory coats for laundering in special plastic bags for collection by contract laundry.

If in doubt, consult your EH&S Compliance Officer or EH&S.

**Use of Respirators**

Respirator use may be necessary in order to maintain exposure levels below permissible limits or short term exposure limits. EH&S can help you determine the necessity for respirator use by evaluating your individual circumstances. Employees may request an evaluation by contacting EH&S or your department's safety committee.

Respiratory protection users must comply with the [ASU Respiratory Protection Plan](#) and includes compliance related to all types of respirators and dust masks. Respirator equipment will be provided at no cost to employees by the specific department.

- Identification of personnel responsible for certain aspects of the CHP
- Information to help employees understand and read labels and MSDSs
- Locations of the MSDSs, lab procedures and other mandated documents
- Physical and health hazards of common chemicals
- Protective procedures from the hazards, e.g., work practices, PPE and emergency procedures
- Methods and procedures to detect the presence of or release of a chemical in the work area (CO monitors etc.)
- Methods and procedures for reporting accidents.

EH&S provides [laboratory safety training sessions](#) at all ASU campus locations. Training registration takes place via EH&S web site.

EH&S can provide specific training for individual departments or principal investigators upon request. Call EH&S to arrange for special training sessions at 480-965-1823.

PIs or Laboratory Managers must provide additional laboratory specific safety training to employees relative to the specific hazards associated in their laboratory (i.e. chemicals and equipment) for Risk Category 3 labs.

PIs or Laboratory Managers must also provide laboratory specific safety training relative to specific hazards associated in their laboratory to non-employees (students) working within their laboratories. Training Record template can be located on the EH&S Form website at <http://cfo.asu.edu/ehs-forms>

Specialized training or training for employees with special needs will be provided to employees when necessary. Please include provisions for employees with special needs within your building and/or department specific emergency preparedness plans. The emergency preparedness plan template is available at <http://cfo.asu.edu/ehs-emergency-plan-template>.

Special needs training can be performed by a department provide it meets the requirements of this CHP. Call EH&S for consultation and approval before conducting such training.

Each employee must complete training as identified on the EH&S Training Determination Table available at <http://cfo.asu.edu/ehs-training-table>. ASU policy [EHS 108-01: Health and Safety Training](#) provides discussion of the required health and safety procedures. This includes initial and annual training for all employees involved in activities covered under this CHP.

New training offered by EH&S includes Compressed Gas Safety and Responsible Party Laboratory Safety Workshop. Look for additional course information in the Training Determination Table.

## **Container Labeling**

Hazardous chemicals in the laboratory must be properly and adequately labeled. PI's and or Laboratory Manager's must assure that all chemicals have labels with legible writing that indicate:

- The label must be written in English using acceptable IUPAC chemical names.
- Name of contents
- Health and Physical Hazard associated with the material. (NFPA hazard warnings, corrosive, oxidizer, acid, alkali, radiation, ect.)
- Concentration
- Date of last peroxide test (if the material is a peroxide former after exposure to oxygen such as with ethyl ether). See Appendix F for more details.

Abbreviations are acceptable on labels only if the laboratory maintains an abbreviation document clearly visible and in the vicinity of the container that indicates the chemical name represented by the abbreviations.

Small containers used for samples with potentially hazardous materials need only be labeled with the sample number or other designation provided a key identifying the sample contents (i.e. water samples in 0.1 N Sulfuric acid) can be easily located by potential emergency responders.

Each container of a hazardous chemical received from the manufacturer with a label must have information that gives:

- The label must be written in English using acceptable IUPAC chemical names.
- Name of content
- Health and Physical Hazard associated with the material. (NFPA hazard warnings, corrosive, oxidizer, acid, alkali, radiation, ect.)
- Name and address of the chemical manufacturer or distributor must also be on the label.
- If a container arrives without the manufacturer's label, an appropriate label must be affixed to it.

Labels must not be removed, except under the following conditions:

- Container is immediately relabeled.
- Chemical in the container is removed, a new type of chemical is placed in the container and the container relabeled with the identity of the new chemical.

### **Special Labeling Practices**

- If it is not practical to label a container, appropriate information may be placed on a sign **next** to the container.
- Chemicals that are time-sensitive or that produce peroxides must be dated indicating the date storage began. See Appendix G of this document for information on common peroxide crystal forming compounds and how to handle them.

Chemical substances developed by the laboratory and for which there is no known written hazard information, use the following procedures:

- If the chemical developed by the laboratory is produced exclusively for the laboratory's use (new compounds and drugs), the laboratory must determine if the substance is hazardous;
- If the substance is hazardous, then the laboratory must label the containers as such, and indicate those hazards on the label;
- If the laboratory is unable to determine the hazards, it must label the chemical as if it were hazardous;
- If the chemical developed by the laboratory is produced for use by another company, the laboratory must develop a MSDS for that chemical substance.

### **Material Safety Data Sheets (MSDS)**

MSDS's must be readily available to laboratory employees for each hazardous chemical used in the work area. The MSDS must contain the following information:

- Chemical and common name
- If a mixture:
  - Chemical and common name of ingredients that are health hazards
  - Chemical and common name of ingredients that are physical hazards
- Physical and chemical characteristics (vapor, pressure, flash point and color)
- Physical hazards, including potential for fire, explosion, and reactivity
- Health hazards, including signs and symptoms of exposure and medical conditions recognized as being aggravated by exposure
- Primary routes of entry into the body
- OSHA Permissible Exposure Limit (PEL), the Threshold Limit Value (TLV), and any other exposure limit used or recommended by the manufacturer
- Indication if the chemical is a carcinogen or potential carcinogen

- Handling procedures including hygienic practices and recommended protective measures during release clean-up
- Personal protective equipment, engineering controls, and work practices
- Emergency and first aid procedures
- MSDS preparation date
- Name, address, and telephone number of the MSDS preparer

All of the above categories must be completed even if no relevant information is found. The same MSDS may be used for several chemicals if they contain similar hazards and ingredients. If additional information concerning a chemical becomes available it must be added to the MSDS within three months.

The responsible party for the laboratory or their designee must maintain a collection of MSDSs for all chemicals in the laboratory and ensure that they are readily accessible to all laboratory employees. The location of the collection must be recorded on the RPI sheet. The location and availability of the collection must be shared with the laboratory employees. The collection can either be maintained as an electronic or paper copy. The collection should include the department's current chemical inventory and MSDSs arranged alphabetically or other manner suitable to locating an MSDS readily.

Note: When MSDS are made available electronically employees must be able to access the MSDS without requesting them directly from another employee and there must be a backup system available in the event of a power failure or computer network connection failure. An example of an acceptable backup system is a computer with a battery back-up and a CD or flash drive with an updated version of the MSDS file kept in a location known to all employees. If a laptop computer is selected as the back-up, measures must be taken to ensure it will not leave the designated location.

### **Guidelines for Transporting Chemicals (including gas cylinders and cryogenic containers)**

- All chemicals should be transported within secondary containers capable of holding all materials in the event of a spill. Transport of any corrosive or heated materials requires secondary containment unless exempted by EH&S. Appropriate personal protective equipment (PPE) is to be used.
- Acceptable secondary containers include plastic bottle carriers with closed tops and handles or non-metal liquid-tight carts with lips on all four sides. Never transport incompatible chemicals in the same secondary containment. Use plastic tubs or separate bottle carriers to prevent incompatibles from mixing.
- Use freight elevators for moving chemicals between floors. If freight elevators are not available, use unoccupied passenger elevators. Stairs should be used only if elevators are not available.

- Wear appropriate Personal Protective Equipment (PPE). Minimum PPE includes safety glasses, lab coat or other appropriate lab attire, and closed toe shoes. Hazardous chemicals must be attended at all times while being transported.
- Individuals transporting chemicals must ensure containers are properly labeled and know what to do in the event of a release or spill. Material Safety Data Sheets (MSDSs) are a good source for this information.
- Transport compressed gas cylinders using special compressed gas cylinder handcarts. The cart should be pushed in front of the transporter and not pulled. When transporting cylinders across asphalt, uneven terrain or between buildings, two people must be in attendance to prevent tipping and unanticipated jolting of the gas cart. Cylinders must be securely attached to the cart and valve protection caps must be in place.
- Materials that are unstable, explosive, or unusually hazardous due to size or toxicity should not be moved without first contacting EH&S (e.g. outdated peroxide formers such as THF, dry Picric Acid, >20 gal containers of flammable or corrosive liquids).
- Transport on paved paths and sidewalks rather than streets or roads. Two people must be in attendance to prevent tipping cart as it is moved over uneven terrain and changes in elevation.
- Before transporting autoclaved materials, please review <http://www.asu.edu/uagc/EHS/documents/autoclave-sop.pdf>.
- Do not transport chemicals in personal vehicles. Transportation must be done only by EH&S or employees authorized to transport hazardous materials. Contact EH&S if a chemical must be transported onto or off of campus.
- Avoid transporting chemicals in a passenger vehicle, but if you must, do not place chemicals in the passenger compartment. Place the containers in the trunk or cargo bed and ensure that they are properly packaged and firmly secured. Never leave chemicals unattended or stored in a vehicle.
- Transport cryogenic materials only in approved storage vessels (e.g. dewar flasks with pressure relief mechanisms). Use appropriate PPE including eye protection in the form of a face shield or goggles, heavy gloves, and closed-toe-shoes. Never transport in the passenger compartment of a vehicle due to the hazard from asphyxiation. Do not transport cryogenic containers with volumes of more than 50 liters in a passenger elevator without setting up a procedure to ensure that no one can access the cab of the elevator during transport.

### **Eating, Drinking, Smoking and Use of Cosmetics**

Many respected institutions including the National Research Council, the Arizona Radiation Regulatory Agency and the Centers for Disease Control and Prevention agree that eating, drinking, smoking, gum chewing, applying cosmetics, and taking medicine in laboratories where hazardous chemicals and materials including unsealed sources of radioactive materials are used must be strictly prohibited. Food, beverages, cups, and other drinking and eating utensils are not to be stored in areas where hazardous chemicals and materials or radioactive materials are handled or stored.

Additionally, contact lenses should not be worn and are not to be handled in locations where hazardous materials are present.

Each Departmental Dean, Director, Chair or their designee may designate areas within laboratory facilities where these activities are permitted. Prohibitions related to the use hazardous materials in these locations must be communicated to all laboratory personnel and the requirement must be enforced.

Refrigerators, freezers, ovens, microwaves and similar appliances in laboratories not intended for use with food or beverage to be used for human consumption must be labeled with the terms “**NOT FOR USE OF FOOD**” or equivalent. Similar appliances in designated locations within laboratories intended for use with food or beverage to be used for human consumption must be labeled “**FOR FOOD USE ONLY**” or equivalent. Areas with refrigerated food for animal use must be labeled as “**FOOD FOR ANIMAL USE ONLY**” or equivalent.

### **Physical Hazards**

Physical hazards associated with each laboratory process must be assessed to determine potential hazards and identify necessary engineering controls, training and required personal protective equipment. Examples of physical hazards include noise, compressed gas uses, explosive or highly reactive chemicals, non-ionizing and ionizing radiation, and potential energy (i.e. springs and hydraulic systems).

Physical hazards are to be assessed during the PPE hazard assessment referenced on page 17 of this plan as part of an overall hazard assessment process. EH&S is available to assist in those evaluations.

All equipment manufacturer’s signage related to physical hazards is to be left intact and employee are to be told to read and adhere to all manufacturer’s warning labels. In situations where the manufacturer’s label has become illegible or missing, physical hazards are to be marked with signage and warnings consistent with the requirements of ANSI Z535.2 Specifications for Accident Prevention Signs and ANSI Z535.1-6 Safety Color Code for Marking Physical Hazards as follows.

Please be sure to verify the following types of equipment are provided with warning labels associated with physical hazards.

- 1) The presence of bare electrical conductors greater than 50 volts as defined in EHS118: Electrical Safe Work Practices.
- 2) Robots, pneumatic lifts, and material handling devices that are not equipped with physical barriers or interlocks engineered to prevent exposure to physical hazards.



Equipment generating excessive noise levels needs to be surveyed by EH&S per the ASU Hearing Conservation Program [http://www.asu.edu/uagc/EHS/documents/asu\\_hearing\\_conservation\\_program.pdf](http://www.asu.edu/uagc/EHS/documents/asu_hearing_conservation_program.pdf) and appropriate signage used to identify where hearing protection may be required or recommended.

Radiation producing equipment and materials, radio-frequency generating equipment and lasers must be labeled. If the equipment manufacturer did not provide labels contact the Radiation Safety Office for assistance at [radiationsafety@asu.edu](mailto:radiationsafety@asu.edu).

Hazards associated with compressed gas are addressed in EHS 122: Compressed Gases <http://www.asu.edu/aad/manuals/ehs/ehs122.html>. This policy requires training for all ASU employees and volunteers that physically handle compressed gas systems.

### **Working Alone Procedures**

Hazard assessments are to be conducted whenever employees and volunteers are working alone as required in [EHS 123 Working Alone with Hazardous Materials, Processes or Equipment](#). Assessments required by this policy should review the means of entry to the workplace and the means of leaving the workplace, the equipment that may be required to be used, the exact tasks that are required to be undertaken, any substances required to be handled, the environment and the atmosphere in which the employee will work alone.

Departments should assess and prioritize the working alone hazards that have been identified and evaluate possible means of elimination or control. Contact EH&S for additional assistance in evaluating working alone hazards.

Examples of controls implemented after the initial hazard assessments included:

- Restricted building access to the building – card key, or “after hours permit” after regular scheduled closing times.
- Department doors are locked when working alone after hours. (Ensure emergency services are able to get into locked buildings).
- Post signage, emergency contact information, and develop a communication system.
- Establish a check-in procedure. Make sure regular contact is kept with all workers. (Personal check by another person, or periodic telephone contact)
- Require the use of a “buddy system” in high-risk situations – ensure that individuals are aware that this option is available to them.
- Where appropriate, consider the use of a security system such as video surveillance cameras, mirrors, observation windows, etc.; however, ensure that informed consent is obtained from employees prior to use.
- Schedule high risk tasks during normal business hours, or when another worker is capable of helping if an emergency situation arises.

Individuals should be trained to increase awareness of methods for identification, hazard reduction and prevention when working alone and dealing with situations or individuals that present a potential risk.

anesthetics should employ waste gas scavenging systems that are connected to non-recirculating exhaust systems.

- Refer to the [ASU Anesthetic Gas Usage](#) program for procedures to minimize exposure to waste anesthetic gases.
- Exhaust systems must be used in conjunction with scavenger systems. Contact EH&S prior to installation of scavenger systems to existing building ventilation.

## **MEDICAL CONSULTATIONS AND MEDICAL EXAMINATIONS**

Employees working with hazardous chemicals will be provided medical attention including any follow-up examinations that the examining physician determines necessary, under the following circumstances:

- Whenever an employee develops signs or symptoms associated with a hazardous chemical exposure which may have occurred in the laboratory
- Where monitoring reveals an exposure level routinely above the action level, or in the absence of an action level, the PEL for an OSHA regulated substance for which there are prescribed exposure monitoring and medical surveillance requirements
- Whenever there is a spill, leak or other release resulting in a potential hazardous chemical exposure of an employee above the PEL or action level
- Examinations will be conducted under the direct supervision of a licensed physician and provided at no cost or loss of pay to the employee.

ASU will provide the physician with the following:

- The identity of the hazardous chemicals to which the employee may have been exposed
- A description of the conditions under which the exposure took place, including any quantitative data if applicable
- A description of the signs and symptoms the employee is exhibiting

After the examination, the physician will submit a written opinion to EH&S that must include the following:

- Any recommendations for medical follow-up
- The results of the medical examination and associated tests
- Any medical condition revealed that would place the employee at increased risk as a result of exposure to a hazardous chemical found in the work place
- A statement by the physician that the employee has been informed of the results of the examination and any medical condition that may require further treatment or examination

- The written opinion will not reveal specific findings of diagnosis unrelated to the occupational exposure.

## **HAZARDOUS WASTE MANAGEMENT**

Laboratory operations that produce waste chemicals are considered as producing hazardous waste. Hazardous waste is regulated by The Arizona Department of Environmental Quality (ADEQ). All laboratory personnel who produce hazardous waste are required to manage their waste according to [ASU's Hazardous Waste Management Compliance Guidelines](#). State and federal law require the university to manage its hazardous waste. Failure to manage hazardous waste properly may result in criminal prosecution and heavy fines.

All laboratory employees who physically place hazardous waste into designated hazardous waste containers are required to complete Hazardous Waste Management training either in classroom or using the web-based training available through EH&S.

### **Broken Glass**

The following is the procedure recommended for handling broken glass. If the broken glass involves blood, microorganisms or bio research materials, please review the following link: <http://cfo.asu.edu/ehs-biowaste-compliance-guideline>.

If a potentially hazardous chemical is involved please review this link: [http://emergency.asu.edu/ep\\_emergency\\_guide](http://emergency.asu.edu/ep_emergency_guide).

If only broken glass is an issue, then the glass should be carefully picked up using forceps or broom and dust pan and placed in a container such as a cardboard box (or other designated substantial container such as a plastic container designated for broken glass) and clearly labeled as broken glass. Please do not place broken glass in ordinary trash containers as it presents a potential risk to those that need to handle it.

## **EMERGENCY PROCEDURES**

Laboratory personnel must be aware of the provisions for emergency procedures and preparedness. Emergency procedures and preparedness include actions or contingencies for:

- Evacuations due to fires, chemical spills, and other situations
- First aid
- Procedures for use of special ventilation areas
- Shut down and lock-out during evacuations
- Location of emergency equipment (showers and eyewashes)

Protocols for handling emergencies are outlined in the [ASU Emergency Response Guide](#) and [Arizona State University Emergency Operations Manual](#). Laboratories must have their own written plan detailing their specific emergency procedures.

## **ACCIDENT AND NEAR MISS REPORTING**

Principal Investigators or Lab Managers must submit accident/near miss reports to EH&S for any accident or near miss situation. Employees will be free from any reprisals for reporting accidents. Accident/Near Miss Reports, corrective actions and suggestions regarding possible improvements can be help safety committees as they strive to improve future laboratory safety.

To report an incident related to an employee, visitor or student in regards to an injury, illness or near miss refer to the [EH&S website](#) and fill out the Accident/Near Miss/Quality Improvement Report. [EHS 115: Incident Reporting and Investigation policy](#)

## **SPILL PREVENTION AND CONTINGENCY**

The basis of a CHP follows the reasoning of the National Environmental Policy Act (NEPA) legislation. NEPA calls for an analysis of the projects impact on the environment, referred to as an environmental impact statement. The purpose of this program is to encourage the laboratory's chemical users to investigate their impact on the environment. The project needs to be reviewed for consequences in the following areas:

- The health and safety of personnel due to chemical use:
- Exposure of the employees to the chemicals, including:
  - Analysis of the need for medical monitoring for employees
  - Training requirements for chemical use in laboratories
- The impact of the research on the property and nearby community:
  - The impact of the products of the research on the hazardous waste disposal program.
  - Efforts in analyzing procedures to reflect pollution prevention mandates.
  - The impact of the research in user fees and/or permitting requirements; and
- An analysis of the energies involved:
  - An emergency contingency analysis, including job hazard analysis or fault analysis

Information discovered by applying EH&S techniques and hazard analysis techniques must be applied by the Laboratory Manager or Principal Investigator in maintaining a safe laboratory.

## Hazardous Materials Compliance

### INTRODUCTION AND SCOPE

Arizona State University's (ASU) academic and research laboratories, along with other university operations, e.g., Facilities Management, generate a variety of hazardous chemical wastes. ASU is classified as a hazardous waste generator by the U.S. Environmental Protection Agency (EPA) and the Arizona Department of Environmental Quality (ADEQ). As a hazardous waste generator facility, ASU is required to comply with a number of hazardous waste laws and regulations. Environmental Health & Safety (EH&S) is responsible for coordinating an effective hazardous waste management program for university facilities. The primary objectives of the program are to protect human health and the environment and insure compliance with university, local, state, and federal hazardous waste regulations. University personnel and facilities involved in activities that generate hazardous waste are also subject to a number of requirements designed to protect personnel, property, and the environment.

University hazardous waste management programs can be somewhat complex and confusing because of the enormous variety of hazardous waste generated; it is the responsibility of each individual university generator to comply with accumulation requirements. The following guidelines have been prepared for university personnel to facilitate proper hazardous waste management and insure compliance with applicable hazardous waste laws and regulations, particularly individual site accumulation requirements.

### REQUIREMENTS

#### Waste Determinations

State and federal hazardous waste regulations specifically require the person who generates a waste to determine if the waste is a hazardous waste by using the following methods:

1. Determine if the waste is listed as a hazardous waste in the regulations; OR
2. If the waste is not listed as a hazardous waste in the regulations, determine if the waste exhibits any of the characteristics of a hazardous waste, i.e., ignitability, corrosivity, reactivity, or toxicity, by either:
  - testing the waste according to approved EPA methods or according to an equivalent approved method; or
  - applying knowledge of the hazard characteristic of the waste in light of the materials or the processes use

For your convenience, **consider all waste chemicals and chemical formulations (abandoned, used, out dated, or otherwise waste-like chemicals and formulations) as a hazardous waste unless the particular waste has been determined not a hazardous waste by EH&S.** Contact EH&S for technical assistance at 480.965.8554.

State and federal regulations regard certain chemicals as "acutely hazardous wastes". These chemicals exist on a list ([see acutely hazardous waste list](#)) and must be handled separately. Chemicals which exist on this list must be reported immediately to EH&S at the point that they become a waste. No more than a total of one quart, at any time, of one or in any combination of all the chemicals on this list are allowed to remain in the laboratory once the chemical(s) become waste.

Whenever there is a doubt about a waste being a hazardous or non-hazardous waste, contact EH&S for technical assistance at 480.965.8554 **EH&S will not accept unknown wastes**, contact EH&S for assistance identifying unknowns. Disposal containers, labeling, hazardous waste tags, segregation, and scheduling of pickups must all be managed in an appropriate manner.

## Disposal Containers

Containers holding hazardous waste must be in good condition, non-leaking and compatible with the waste being stored. The container must always be closed during storage, except when it is necessary to add or remove waste. Hazardous waste must not be placed in an unwashed container that previously held an incompatible waste or material. Incompatible wastes must not be mixed or stored in the same container. If a container holding hazardous waste is not in good condition, or if it begins to leak, the generator must transfer the waste from this container to a container that is in good condition, or manage the waste in some other way that prevents a potential for a release or contamination. A storage container holding a hazardous waste that is incompatible with any waste or other materials stored nearby in containers must be separated from the other materials or protected from them by means of a partition, wall, or other device. One gallon glass hazardous waste containers with screw top lids are available at no charge to university personnel from EH&S. Also, the original chemical container can serve as a waste container as long as the above requirements are met. Containers may exceed one-gallon as long as they meet the above stated requirements. However, containers in excess of five gallons must have prior approval from EH&S before being used as a hazardous waste storage container. Contact EH&S to check the availability of larger containers.

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## Container Labeling

While hazardous waste is being accumulated, the container holding the waste must be marked with the words "**HAZARDOUS WASTE**" and with words that identify the contents of the container. For the purpose of waste determination, a complete inventory of wastes being accumulated in the container must be kept with the container. This can be accomplished by using hazardous waste tags and labels available through EH&S. If the original container is to be used for waste disposal, the words "**HAZARDOUS WASTE**" shall be legibly written with a permanent marker above or next to the chemical name. If the container is to be used for a waste different from the original contents, the original label must be removed or rendered illegible and a new label placed on the container. Again, hazardous waste must not be placed in an unwashed container that previously held an incompatible waste or material. The container must also be marked with the hazard associated with the waste e.g., flammable, corrosive, toxic, etc.

## Hazardous Waste Tags

Hazardous waste tags must be filled out by the waste generator. The waste tags are used by EH&S personnel to identify waste containers and determine waste compatibilities. Improper or inaccurate tagging could present a serious safety threat to EH&S personnel handling these wastes. EPA and ADEQ review the tags to determine the university's compliance with hazardous waste regulations. Inadequate or incorrect information on waste tags may result in the container not being picked up. Therefore, in the interest of efficiency, please make certain the waste tags are complete, accurate, and legible to avoid having to reschedule a pick-up. The following information must be included on the tags:

- **Chemical Name:** Use full chemical name, not formulas. Product names or trade names are acceptable if the manufacturer's name and address or a material safety data sheet can be supplied with the material. Also, indicate the concentration of the chemical used in percent, molarity, ppm, etc.
- **Amount:** Give total volume or weight of each chemical in the container.
- **Volume %:** List percentage of the total volume of each chemical contained if more than one compound is being accumulated in the waste container.
- **Generator:** List name of the individual responsible for preparing the waste and completing the

waste tag.

- **Phone:** Supply a telephone number at which the generator can be reached during normal university hours (8am - 5pm).
- **Date:** Mark the date on which the waste container is ready for pick-up.
- **Department:** Identify the university department with which the generator is affiliated.
- **Building/room:** List physical location in which the waste is being held for collection.
- **Category:** Choose the appropriate category (hazard) descriptions from the list below and write it in the category block. Include other hazards (e.g., "carcinogen") as well.
  - Radioactive material
  - Corrosive material (solid)
  - Poisonous compressed gas
  - Poison
  - Flammable gas
  - Non-flammable gas
  - Combustible material
  - Flammable liquid
  - Pyrophoric
  - Oxidizer
  - Self-reactive
  - Flammable solid
  - Spontaneously combustible
  - Corrosive material (liquid)
  - Water-reactive
  - Explosive
  - Irritant
  - Biohazard/infectious
- **pH:** The pH of the solution is required for aqueous wastes. pH can be taken using pH paper or a pH meter.

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### Segregation

As previously stated, all chemicals are to be considered hazardous waste when disposed. It is the policy of the university that no waste be disposed or discharged into the sanitary sewer. Whenever possible, chemical wastes should be collected and segregated by the following categories to ensure safe handling and to expedite disposal:

- Bases, caustics and cyanides
- Explosive and shock sensitive materials - Examples: picric acid, perchloric acid, dinitrophenyl hydrazine
- Flammable liquids - non-halogenated organic solvents
- Flammable solids - Examples: camphor, naphthalene
- Gases - Examples: lecture bottles, small non-returnable cylinders
- Inert materials - Examples: asbestos and silica
- Inorganic acids - Examples: hydrochloric acid, hydrofluoric acid (collect separately in plastic container), sulfuric acid, nitric acid, phosphoric acid
- Metal salts and heavy metals, (collect heavy metals separately)
- Non-flammable organic compounds - Examples: halogenated organic solvents, glycerol, aniline;



- Organic acids - Examples: formic acid, acetic acid
- Oxidizers - Examples: nitrates, permanganates, perchlorates, nitric acid over 40% (collect separately), chromic acid "cleaning solution" (collect separately)
- Peroxidizers, including peroxidizable solvents
- Pesticides and herbicides
- Polychlorinated biphenyls (PCB's) and dioxins - accumulate separately
- Polymerizables
- Radioactive materials - these are handled by the Office of Radiation Protection 480.965.6140
- Reactive materials (react when exposed to water, air, or friction), Examples: alkali and alkaline earth metals, acid halides, phosphorous pentoxide, thionyl chloride

## **SCHEDULING AND WASTE PICK-UP**

When the waste container is ready for pick-up (Note: Do not fill waste container beyond the bottom of the neck of the container) and the waste tag has been completed, use the on-line hazardous [waste pickup request](#) or the link located on our [website](#) This will initiate the waste pick-up process. Waste is routinely picked up throughout the campus Monday through Friday. If you have waste in one location which totals more than 55 gallons in overall amount, or if you have a total of one quart of all acutely hazardous waste, please note that in the comments section of the request. Include the hazardous waste tag numbers on the pickup request.

## **WASTE OIL**

Waste oil is collected and recycled. However, waste oil must be kept as uncontaminated as possible. EH&S requires oil to be kept separate from other chemicals, particularly solvents, metals, and pesticides. If the oil is contaminated, indicate so on the hazardous waste tag and it will be managed and disposed of in an approved manner.

## **PENALTIES**

Severe civil and criminal penalties can be imposed upon the generator and university for irresponsible and illegal hazardous waste management and disposal practices. These procedures have been designed to properly and economically manage the university's hazardous waste while complying with all applicable federal, state and local regulations. EH&S is available for technical assistance and encourages all university personnel to utilize the service. Please contact EH&S if you have any questions regarding responsible hazardous waste management or need additional information or assistance in managing your hazardous waste.

## **APPLICABLE REGULATIONS**

40 CFR Parts 260 through 271  
A.A.C. R18-8-260 et seq.

## **UNIVERSITY DOCUMENTATION AND MANUALS**

[Arizona State University Chemical Hygiene Plan](#)

Arizona State University Pollution Prevention Plan

Arizona State University Hazardous Waste Accumulation Facility Emergency Contingency Plan

Research and Sponsored Programs Policies and Procedures [RSP 105](#) Hazardous Chemical Waste

## **TECHNICAL CONTACT**

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# **Compressed Gases Safety Program**

**Arizona State University  
Department of Environmental Health & Safety  
Revision Date: April 2010**

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**Arizona State University**  
**Environmental Health & Safety**  
**Compressed Gases Safety Program**

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**Arizona State University  
Environmental Health & Safety  
Compressed Gases Safety Program**

**Introduction and Purpose**

Arizona State University continually strives to provide a learning, teaching, and research environment free from recognized hazards. The University requires the safe handling, use and storage of compressed gas cylinders to protect employees and students from potential physical and health hazards associated with using compressed gases in laboratories or other location that are part of the University.

**Scope and Application**

Arizona State University wants to ensure employees who handle compressed gases understand the health and physical hazards of the compressed gas cylinders, the contents, proper handling, use, storage, and emergency procedures. To accomplish this, the (EH&S) Environmental Health and Safety Department will ensure compliance to the Occupational Safety and Health Administration (OSHA) Compressed Gases in 29CFR, Subpart H Hazardous Materials 1910.101 to 1910.106 and the Compressed Gas Association (CGA) requirements.

Compressed gas cylinders can present a variety of hazards due to their pressure and /or contents. This program covers requirements which must be followed for the use of all compressed gases. In addition to the standard required work practices for inert gases, hazardous gases may require additional controls and work practices including, but not limited to, the use of gas cabinets, gas monitors, emergency shutoffs, proper equipment design, leak testing procedures, and the use of air supplying respirators for certain highly toxic gases.

This program applies to the storage, use, and handling of gases in pressurized portable containers and gas systems. The primary focus of this program is on single gas uses and systems. Additional requirements may be applied to:

- Use of multiple gases in a single control area or building
- Large compressed gas facilities, storage areas, or use areas

**Responsibilities**

Deans, Directors, and Chairs

- Deans, directors, and chairs are responsible for establishing and implementing department information and training programs for their respective areas. Delegation of this responsibility to the Principle Investigator (PI), laboratory supervisor or manager or Compliance Officer and/or safety committee is acceptable.

### Principal Investigator/Laboratory Manager

- The laboratory supervisor is the Principal Investigator (PI) assigned to the laboratory. It is the responsibility of each PI to understand the processes and hazards in the work area.
- Ensures that University policies are enforced and safe work practices are followed.
- Provides for and acquire adequate instruction in the use and maintenance of compressed gas cylinders for employees.

### Environmental Health & Safety (EH&S)

- Ensures that University policies are enforced and safe work practices are used.
- Assists, advises and provides training as necessary.
- Reviews and approves procedures for all controlled, highly toxic or hazardous gases.
- Assists, advises and instructs University employees in the care and handling of compressed gas cylinders and gas systems.

### Compliance Officers

Arizona State University's Environmental Health & Safety Management Policy (EH&S MP) calls for the University to be a model of quality in environmental health and safety. Compliance Officers are critical links in the development of this level of quality. Compliance Officers undergo special training and can be a crucial step in maintaining compliance to EH&S programs.

- Assist and review compressed gas storage and installation to ensure safe work practices are used.
- Coordinate activities between compressed gas uses and the EH&S department.

### ASU Employees

- Performs all work with compressed gases in accordance with ASU policies and prudent safe work practices.
- Obtain required training to safely work in the laboratory area.

### **Training Requirements and Competency Assessment**

All persons handling or using cylinders must have basic training initially at time of employment and periodically thereafter. Training will include review of information on the hazards associated with compressed gas include oxygen displacement, fires, explosions, toxic effects from certain gases and the physical hazards associated with pressurized systems. Special storage, use and handling precautions necessary to control these hazards will also be covered.

The training program will be provided by EH&S or a Lab Manager and will include classroom instruction and operational training on specific compressed gas cylinder hazards on campus.

Employees will require refresher training under any of the following conditions:

- Changes in the workplace rendering previous training obsolete.
- Changes in the types of cylinder systems or equipment used that would render previous training obsolete.

Inadequacies in an employee's knowledge of compressed gas cylinders or equipment or observed behavior indicate that the employee has not retained the required training.

### **Personal Protective Equipment**

- Safety Glasses: Use especially when connecting and disconnecting gas regulators and lines.
- Foot Protection: Use when moving or transporting cylinders.
- Gloves and Clothing: To protect against frostbite, corrosives and pinch points
- Face protection: Face shield shall be worn when there are additional hazards to the face.

### **Labeling Requirements**

Compressed gas cylinders shall be legibly marked for the purpose of identifying the gas content with either the chemical or the trade name of the gas. Such marking shall be by means of stenciling, stamping, or labeling, and shall not be readily removable. Whenever practical, the marking shall be located on the shoulder of the cylinder (OSHA Standard 29 CFR 1910.253 (b) (1) (ii)).

- A durable label should be provided that cannot be removed from the compressed gas cylinder.
- Compressed gas cylinders that do not clearly identify its contents by name should not be accepted for use.
- Color-coding is not a reliable means of identification; cylinder colors vary from supplier to supplier, and labels on caps have no value because many caps are interchangeable.
- Tags should be attached to the gas cylinders on which the names of the users and dates of use can be entered.
- If the labeling on the gas cylinder becomes unclear or defaced so that the contents cannot be identified, the cylinder should be marked "contents unknown" and the manufacturer must be contacted regarding appropriate procedures for removal.

## Proper Storage of Compressed Gas Cylinders

All compressed gas cylinders must be properly stored in compliance with OSHA, and NFPA code requirements. Cylinders internal pressure can reach over 2,000 psi. In the event of a container breach, the cylinder becomes a projectile hazard.

Signage required at compressed gas cylinder storage locations includes:



The following precautions must be taken for the storage of compressed gas cylinders.

1. Cylinders must be stored in a dry, cool, well-ventilated secure area.
2. All cylinders whether empty or full must be stored upright and secured by chains, straps or in racks to prevent them from falling.
3. Segregated cylinders by contents. For example, flammable gases must be stored separately from oxidizing gases by a distance of 20 feet or a 5 foot high, one-hour fire-rated wall.
4. Prevent smoking or open flames in oxidizer or flammable gas storage areas
5. Do not expose cylinders to corrosive materials such as corrosive gas or other combustible materials.
6. Segregate full and empty cylinders, use first in first out inventory control method.
7. Store cylinders away from heavily traveled areas and emergency exits.
8. Provide adequate access for cylinder handling and material handling carts.
9. Visually inspect stored cylinders on a routine basis, look for indication of leakage or problems.
10. All cylinder storage areas, outside or inside, shall be protected from extreme heat and cold and from access by unauthorized personnel. Prevent indoor or outdoor temperatures from exceeding 125<sup>0</sup>F or 52<sup>0</sup>C.

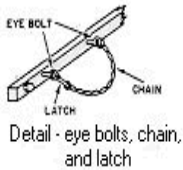
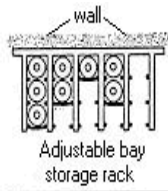
## Securing Compressed Gases Cylinders

Cylinders must be secured in one or more of the following ways:

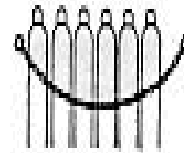
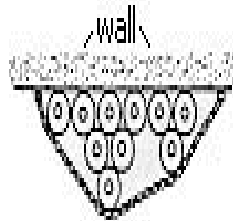
- \_\_\_ By a noncombustible, two-point restraint system (e.g., chains) that secures the cylinder. Nesting of cylinders is not an approved method that can be used to secure cylinders. Individual cylinders can use a bracket or saddle to support the cylinder.
- \_\_\_ By a noncombustible rack, framework, cabinet, approved strapping device, secured cylinder cart, or other substantial assembly that prevents the cylinder from falling.
- \_\_\_ Straps must surround the cylinder approximately 1/2 to 1/3 the height of the cylinder measured from the floor.
- \_\_\_ Gas cylinders must be secured to prevent falling due to accidental contact or vibration.



### Recommended

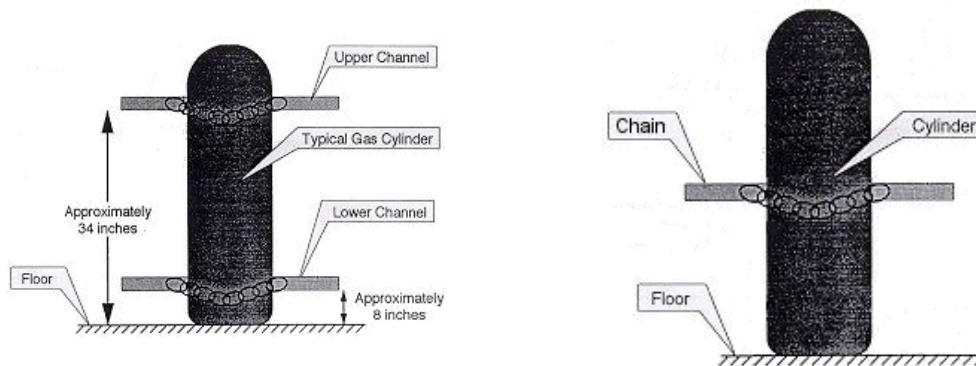


### Not Recommended



Cylinders not adequately supported





## METHODS OF SECURING CYLINDERS

### Proper Handling of Compressed Gas Cylinders

Compressed gas cylinders should be handled only by those familiar with the hazards and who can demonstrate safety precautions working with cylinders. Cylinders are heavy and awkward to move and improper handling can result in sprain, strain, falls, bruised, or broken bones. Other hazards such as fire, explosion, chemical burns, poison, and cold burns could occur due to mishandling. Eye protection and substantial footwear should always be used when transporting compressed gas cylinders. Always push cylinder carts, never pull.

The following precautions must be taken when handling compressed gas cylinders.

- Wear the appropriate personal protective equipment when handling cylinders.
- Cylinders must always be transported on wheeled cylinder carts with retaining straps or chains.
- Do not roll or drag a cylinder over a few feet necessary to position the cylinder.
- Compressed gas cylinders must be transported with protective caps in place. Do not lift the cylinder by the protective cap.
- Avoid dropping the cylinder; do not tamper with pressure-relief devices or removing any product label or shipping hazard labels.
- Don't try to catch a falling cylinder.
- Do not allow grease or oil to come in contact with oxygen cylinder valves, regulators, gauges or fittings. An explosion or fire can result. Oxygen cylinders and apparatus must be handled with clean hands and tools.
- Open cylinder valve slowly, directed away from your face.
- Do not attempt to refill compressed gas cylinders; this is only to be done by qualified manufacturer of compressed gases.

ASU Compressed Gas Compliance document can be found can be found at [ASU Compressed Gas Cylinders Guidelines](#) .

## Proper Use of Compressed Gas Cylinders

Take the following precautions must be used to prevent injuries caused by the improper use of compressed gases.

- Know and understand the gases associated with the equipment being used.
- Use regulators approved for the specific gas.
- Do not mix gases in a cylinder.
- Do not permit cylinders to become part of an electrical circuit.
- Use non-sparking tools (brass) when working with flammable/explosive materials.
- Prevent sparks and flames from contacting cylinders.
- Never strike an arc on a cylinder. Never introduce another product into the cylinder.
- Do not discharge the contents from any gas cylinder directly towards any person.
- Do not force cylinder valves connections that do not fit.
- Open cylinder valve slowly and carefully after the cylinder has been connected to the process. Use check valves to prevent reverse flow into the cylinder.
- Closed the cylinder valve and release all pressure from the downstream equipment disconnected the cylinder anytime there an extended non-use period is expected.
- Following storage and handling requirements.
- Never use a compressed gas in any confined space.
- Never work alone when using compressed gas.
- Never use compressed gas to dust off clothing.
  - This could cause injury to the eyes or body and create a fire hazard. Clothing can become chemically saturated and burst into flames if touched by an ignition source such as a spark or cigarette.

If the cylinders valve does not operate properly, do not attempt to force the valve to turn. The cylinder should be returned to vendor or Lab Stores. Employees must not attempt to repair cylinders or cylinder valves, or to force stuck or frozen cylinder valves.

## Cylinder Size

Use lecture sphere or bottle size hazardous gas sources in a returnable cylinder when small volumes are needed.

While the initial purchase cost per cubic foot may be lower when hazardous gases are purchased in full sized cylinders the overall cost of experimental setup which may require local ventilation, gas cabinets, stainless steel piping and purging systems may offset the apparent saving from buying hazardous gases in full sized cylinders

	High Pressure Cylinders							
	R	RR	Q	LD	S	K	T	KHP
Size								
Height (In)	14	17	32	43	47	51	55	51
Weight (Lbs)	11	24	46	58	61	113	139	188
Nominal Volume (CU FT)	20	40	80	122	150	244	330	N/A

## Tubing and Piping Connections

Hazardous gases must be dispensed using systems that are properly cleaned and compatible with the gas in use. Bust pressure of tubing and piping must be twice the maximum pressure on the second stage regulator. Exceptions to this requirement may be made for short sections of tubing when it and the compressed gas cylinder are completely enclosed in a fume hood and low pressures and flow rates are used.

- Use "hard" piping (such as copper and stainless steel tubing) whenever possible (as opposed to flexible or plastic tubing). Never use cast iron pipe or fittings.
- Teflon tape should never be used on cylinder connections or tube-fitting connections. Use Teflon tape only on pipe threads where the seal is made at the threads. All other connections have metal to metal face seals or gasket seals.
- When flexible tubing must be used, select tubing compatible with the chemical and pressure properties of the gas being used in the system. Do not use flexible tubing for highly toxic gases. Flexible tubing should only be used within "line of sight." Do not run flexible tubing through walls, ceiling spaces, doorways, or other non-visible pathways if chafing is likely to occur.
- Always clamp flexible tubing connections. Use a clamp approved for the maximum allowable pressure that the connection is subject to. Never use wire, which may cut the flexible tubing.
- Most flexible tubing deteriorates with age or exposure to chemicals or UV light. Replace old flexible tubing before it deteriorates.
- Always leak-check tubing or piping connections when using hazardous gases.
- Secure and support tubing or piping to keep it in place and to prevent "whipping" if a connection fails under pressure.
- Appropriately rated flexible lines are suitable for manifold/cylinder connections.

## **Regulators**

Regulators reduce high pressure gas on a cylinder or process line to a lower usable level. Regulators provide additional safety measures by preventing fire/explosions, chemical or cold burns, poisoning and system over-pressurization.

Safety considerations include materials of construction to ensure chemical compatibility, and never use any regulator for gases other than those for which it is intended.

Care must be taken when used left-handed threaded connectors. Do not force connected or over tighten connection. Check the bolt for hash marks indicating a left-handed threaded connection.

## **Valves on Compressed Gas Cylinders**

Most compressed gas cylinders require the installation of at least one valve. This valve allows the cylinder to contain gases and allows gas to be filled into or emptied from the cylinder. The cylinder valve is the most vulnerable part of the compressed gas cylinder. Leaks can also occur at the regulator, cylinder stem and at the hose connection.

**Types of Valves** Check valves are mechanical valves that permit gases and liquids to flow in only one direction, preventing process flow from reversing. Common types of valves include check, ball, disk, butterfly, gate, diaphragm, needle and solenoid. Valves can be made of plastic, stainless steel or other material. Valves serve unique requirements so it is important to select the specific type of valve for your operation.

Precautions to consider while using valves are:

- \_ Open valves slowly to control pressure surges and heat of compression.
- \_ Inspect the valve for damage and foreign materials before connecting to the cylinder.
- \_ Never use a damaged valve where integrity may have been affected. Discontinue the use a valve that operates abnormally, i.e., becomes noisy or progressively harder to operate.
- \_ Never tamper with regulatory or attempt to tighten or loosen the valve into or out of the cylinder.
- \_ Never use an automatic operator, adapter, wrenches, or other tools to obtain a mechanical advantage on hand wheel-operated valves without reviewing all safety requirements.
- \_ Never lubricate valves or their connections.
- \_ Never drag, lift, or move a cylinder using the valve or the hand wheel as a handle.
- \_ Use the cylinder valve to regulate flow or pressure.
- \_ Move cylinders without the transport cap installed.

## **Restrictive Flow Orifices (RFO'S)**

Restrictive Flow Orifices are installed in the cylinder valve outlet and provides significant safety benefits for uses of hazardous gases including pyrophoric gases like silane. Consult the EH&S Office for additional information on RFO's.

## **Rupture Disk**

A rupture disk is a non-reclosing pressure relief device that protects a pressure vessel like a compressed gas cylinder from over pressurization or potentially damaging vacuum conditions. A rupture disc (also known as a rupture disk or bursting disc), is designed to provide a leak-tight seal within a pipe or vessel, until the internal pressure rises to a predetermined level. At that point the rupture disc bursts preventing damage to the equipment from overpressure.

## **Vacuum Pumps**

Hydrocarbon based vacuum pump oil is incompatible with strongly oxidizing and many reactive gases. New vacuum pumps that have inert lubricants such as DuPont Krytox and never contained oil-based lubricants must be used with oxidizing and reactive gases.

Vacuum pumps must be securely vented to a fume hood or other approved exhaust system with tubing that is compatible with the gases used. Exhaust lines must be as short as feasible. Vented enclosures may be required for vacuum pumps depending on the toxicity of the gases used.

## **Specific Requirements of Compressed Gas Cylinders**

Read the label on the cylinder and identify the contents before using. If the label is illegible or missing, return the cylinder to the supplier. Don't rely on stenciling or color of the cylinder. Do not use a cylinder with unidentified contents. All cylinders must be permanently labeled as to their contents and if they are full or empty (example - an empty cylinder may be marked "MT").

Empty cylinders must also be separated from full cylinders. Know the hazards of the contents and follow appropriate safe use practices for the material inside. Refer to the specific Material Safety Data Sheet (MSDS) for additional information.

Gases that have poor warning properties such as colorless, odorless, tasteless or non-irritating or used in enclosed areas must be reviewed for exposure hazards. General or mechanical ventilation, personal badges, hand held monitors or sensors, room monitors and/or environmental monitors should be considered. Delivery systems must be designed with auto shut-offs.

## **Types of Compressed Gases**

The types of compressed gas can be divided into three categories, each with unique characteristics.

**Liquefied Gas** can become liquid at normal temperatures when they are inside a cylinder under pressure. When gas is removed from the cylinder, enough liquid evaporates to replace it, keeping the pressure in the cylinder constant. Common examples include anhydrous ammonia, chlorine, propane, nitrous oxide and carbon dioxide.

**Non-Liquefied** is also a compressed, pressurized or permanent gas. These gases do not become liquid when they are compressed at normal temperatures or even very high pressures. Common examples are oxygen, nitrogen, helium and argon.

**Dissolved Gas** can also be compressed. A common example of dissolved gas is acetylene. Care should be taken when using acetylene or welding. Consult your supervisor before using acetylene.

## **Cryogenic Liquids**

All cryogenic liquids should be used with caution due to the potential for skin or eye damage due to the low temperature, and the hazards associated with pressure buildups in enclosed piping or containers. Special hand/arm protection includes the use of cryogenic gloves; these gloves should be loose fitted and are designed to protect human tissue from cold burns.

Personal protective equipment includes a full face shield, full foot cover and clothing that prevent the absorption of liquids such as thick sweater, jackets and pants with cuffs for transferring cryogenic fluids.

Portable containers should only be used where there is sufficient ventilation. Do not place containers in a closet or other enclosed space where there is no ventilation supply to the area. The buildup of inert gas in such an area could generate an oxygen deficient atmosphere.

Special vacuum jacket containers with loose fitting lids should be used to handle small quantities. Vacuum jacketed containers provided by the gas supplier will have overpressure relief devices in place.

Any space where cryogenic fluids may accumulate (consider leakage into enclosed equipment) must be vented or protected by overpressure relief devices. Tremendous pressures can result in enclosed spaces as the liquid converts to gas. For example, one cubic centimeter of liquid nitrogen will expand to 700 times this volume as it converts (warms) to its gaseous state.

Containers to be filled with cryogenic liquids should be filled slowly to avoid splashing.

Cryogenic containers showing evidence of loss of vacuum in their outer jacket (ice buildup on the outside of the container) should not be accepted from the gas supplier. Contact with air (or gases with a higher boiling point) can cause an ice plug in a cryogenic container.

### **Dewar Safety**

See Appendix B on Dewar Safety

### **Inerts, Oxidizers, Pyrophoric or Toxic Compressed Gas Cylinders**

Consult the Material Safety Data Sheet for all gases. Some gases are pyrophoric (phosphine) corrosive (hydrogen chloride), toxic (ethylene oxide), anesthetic (nitrous oxide), or highly reactive (anhydrous ammonia). Call EH&S at 480-965-1823 if you are unsure how to control the dangerous properties of a particular compressed gas.

**Inerts** - Inerts such as Nitrogen is a gas that makes up about 78% of the air we breathe. Nitrogen is a dry, inert, colorless and odorless gas; it is nonflammable and noncorrosive. Even with these somewhat minor hazard characteristics, Inert gases cause numerous emergency incidents each year. Care must be taken to prevent oxygen deficient atmosphere, Equipment that used Nitrogen or other inerts should use mechanical ventilation or exhaust monitoring to prevent asphyxiation.

- Carbon Dioxide must be treated with caution. If left to leak into closed space, these gases may displace oxygen and create a risk of asphyxiation.

### **Oxidizers**

- Oxidizing gases such as compressed oxygen or nitrous oxide, while not combustible themselves, will cause many materials to burn violently. Never use grease, solvents, or other flammable material on an oxygen valve, regulator, or piping.
- Anesthetic gas may causes loss of sensation with or without the loss of consciousness and the storage handling and use of these type materials must follow these requirements.

### **Flammables**

- Flammable gases such as propane, hydrogen, and acetylene always have a red label. However, the color of the cylinder itself is not a good indicator of flammability as different distributors may use different colored cylinders for the same gas. Check the label for flammability.

- The flammable range of a gas, including all concentrations in air between the Lower Flammable Limit (LFL) and the Upper Flammable Limit (UFL). An example: Hydrogen LFL = 4%; UFL = 75%.
- The auto-ignition temperature is the minimum temperature that gas and its vapors can spontaneously ignite in air. Examples include Silane or Diborane.
- Flammable gas must be segregated from oxidizers.

**Pyrophoric** - hazardous gas (arsine, silane, phosgene, diobrane, etc.) cylinders should be stored in a suitable exhausted location. If a hazardous gas cylinder develops a leak, evacuate and restrict area access.

**Toxic** - gas use may require the use to toxic gas monitoring, handling, storage and emergency procedures as well as additional facility considerations. A Process Hazard Assessment must be completed before a toxic gas can be used. Specific operating procedures must be developed and reviewed annually per the Chemical Hygiene Plan. Contact EH&S if you plan to use these gases.

**Reporting Requirements** - Maintain constant awareness of and respect for compressed gas cylinders and equipment. Comply with all applicable ASU safety rules and compliance with all applicable ASU EH&S rules are mandatory.

- Report all suspected leaks immediately. If the material in the tank is highly toxic or flammable and you suspect a leak, evacuate everyone out of the area and report it to the appropriate person in your department.
- Employees shall report any safety concerns to their supervisor or EH&S.
- Accidents must be reported and any injury must follow ASU injury reporting requirements.
- Representatives of the EH&S Office are authorized to issue warnings to employees and stop unsafe work from continuing.

### **Compressed Gas Cylinder Emergencies**

- Emergencies involving compressed gases are unlikely, provided the recommendations are followed for their correct storage, handling, and use. When problems do arise they are usually due to: fire threatening the cylinder, toxic or inert gas leaks, or unplanned chemical or other reaction.
- Most leaks occur at the valve and valve stem fitted on the top of the cylinder. Leakage here is frequently due to dirt in the connection, or damaged connections or washers where required. Such leaks are easily rectified. Attempt to tighten the connection.
- If cylinders are involved in any type of an emergency, and it's safe to do so, isolate the gas outdoors and away from sparks and heat. In any event all defective cylinders should be clearly labeled and returned to the supplier.



## **Appendix A**

### **Definitions**

**Anesthetic gas** - A gas that may causes loss of sensation with or without the loss of consciousness.

**CGA** – Compressed Gas Association

**Corrosive Gas** - A gas that can cause visible destruction of, or irreversible alterations in, living tissue (e.g., skin, eyes, or respiratory system) by chemical action.

**Cryogenic Liquids** – Gases condensed to liquid form at extremely low temperatures. Example: Liquid Nitrogen is  $-196^{\circ}\text{Celsius}$  ( $-320^{\circ}\text{Fahrenheit}$ ). The term “cryogenics” applies to all temperatures less than  $-150^{\circ}\text{C}$  ( $-238^{\circ}\text{F}$ ).

**Compressed Gas** -

- (i) A gas or mixture of gases in a container, having an absolute pressure exceeding 40 psi at  $70^{\circ}\text{F}$  ( $21.1^{\circ}\text{C}$ ) or
- (ii) A gas or mixture of gases in a container, having an absolute pressure exceeding 104 psi at  $130^{\circ}\text{F}$  ( $54.4^{\circ}\text{C}$ ) regardless of the pressure at  $70^{\circ}\text{F}$  ( $21.1^{\circ}\text{C}$ ) or
- (iii) A liquid having a vapor pressure exceeding 40 psi at  $100^{\circ}\text{F}$  ( $37.8^{\circ}\text{C}$ ) as determined by ASTM D-323-72.

**Corrosive or Corrosive Material** - As defined by the Department of Transportation (DOT), a corrosive material is a liquid or solid that causes visible destruction or irreversible alterations in human skin tissue at the site of contact or in the cases of leakage from its packaging, a liquid that has a severe corrosion rate on steel

**DOT** - U.S. Department of Transportation.

**Flammable gas** - A gas that can be ignited in air.

**Flammable** - A chemical that falls into one of the following categories:

- (i) Aerosol, flammable means an aerosol that, when tested by the method described in 18 CFR 1500.45, yields a flame projection exceeding 18 inches at full valve opening, or a flashback (a flame extending back to the valve) at any degree of valve opening
- (ii) Gas - flammable:
  - (A) A gas that at ambient temperature and pressure, forms a flammable mixture with air at a concentration of 13 percent by volume or less or
  - (B) A gas that, at ambient temperature and pressure, forms a range of flammable mixtures with air wider than 12 percent by volume, regardless of the lower limit.

(iii) Liquid, flammable: Any liquid having a flashpoint below 100 F (37.7°C), except any mixture having components with flashpoints of 100 F (37.7°C) or higher, the total of which make up 99 percent or more of the total volume of the mixture.

(iv) Solid, flammable: A solid, other than a blasting agent or explosive as defined in § 1910.109(a), that is liable to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a serious hazard. A chemical must be considered to be a flammable solid if, when tested by the method described in 16 CFR 1500.44, it ignites and burns with a self-sustained flame at a rate greater than one-tenth of an inch per second along its major axis.

**Flammable limits** - The concentration of flammable vapor in air, oxygen, or other oxidants that will propagate flame upon contact when provided with a source of ignition. The lower explosive limit (LEL) is the concentration below which a flame will not propagate; the upper explosive limit (UEL) is the concentration above which a flame will not propagate. A change in temperature or pressure may vary the flammable limits.

**Flashpoint** - The minimum temperature at which a liquid gives off a vapor in sufficient concentration to ignite when tested as follows:

(i) Tagliabue Closed Tester (See American National Standard Method of Test for Flash Point by Tag Closed Tester, Z11.24-1979 (ASTM D 56-79))-for liquids with a viscosity of less than 45 Saybolt Universal Seconds (SUS) at 100°F (37.8°C), that do not contain suspended solids and do not have a tendency to form a surface film under test or

(ii) Pensky-Martens Closed Tester (see American National Standard Method of Test for Flash Point by Pensky-Martens Closed Tester, Z11.7-1979 (ASTM D 93-79))-for liquids with a viscosity equal to or greater than 45 SUS at 100°F (37.8°C), or that contain suspended solids, or that have a tendency to form a surface film under test or

(iii) Setaflash Closed Tester (see American National Standard Method for Test for Flash Point by Setaflash Closed Tester (ASTM D 3278-78)).

Organic peroxides, which undergo auto accelerating thermal decomposition, are excluded from any of the flashpoint determination methods specified above.

**Hazardous gas** - A gas that is included in one or more of the following hazard categories: corrosive, flammable, health hazard, oxidizer, pyrophoric, reactive, or toxic.

**Health Hazard** - Any chemical for which there is at least one scientific study that shows it may cause acute or chronic health symptoms. This includes chemicals which are carcinogens, toxic or highly toxic, irritants, corrosives, sensitizers, or chemicals that effect target organs including the lungs, kidneys, nervous system, pulmonary system, reproductive system, skin and eyes.

**Ignition Source** - Anything that provides heat, sparks, or flame sufficient to cause combustion/explosion.

**Inerts** – Gases that do not readily reactive with other elements; forming few or no chemical compounds.

**Incompatible** - Materials which could cause dangerous reactions from direct contact with one another are described as incompatible.

**Laboratory** – A laboratory is defined as a facility or room where the use of potentially hazardous chemicals, biological agents or sources of energy (i.e. lasers, high voltage, radiation, etc.) used for scientific experimentation, research, or education.

**LEL** – (Lower Explosive Limit) LEL is the lowest concentration of a gas or vapor in the air that can produce ignition or explosion.

**Mass Flow Controller-** (MFC) is a device used to measure and control the flow of gases.

**MSDS** – (Material Safety Data Sheet) Written or printed material about a chemical that specifies its hazards, safe use and other information. It is prepared by the chemical manufacturer, and is required by federal law.

**Mechanical Exhaust** – Mechanical exhaust systems use a powered device, such as a motor-driven fan or air/street venturi tube, for exhausting contaminants from a workplace, vessel, or enclosure.

**NFPA** - National Fire Protection Association.

**OSHA** – (Occupational Safety and Health Administration of the U.S. Department of Labor)

**OSHA** - is a federal agency with safety and health enforcement authority for most of U.S. industry and business.

**Oxidizer** - Department of Transportation defines oxidizer or oxidizing material as a substance that yields oxygen readily to stimulate the combustion (oxidation) of organic matter. Chlorate ( $\text{ClO}_3$ ), permanganate ( $\text{MnO}_4$ ) and nitrate ( $\text{NO}_3$ ) compounds are examples of oxidizers.

**Oxidizing gas** - A gas that initiates or promotes combustion in materials, either by catching fire itself or by causing a fire through the release of oxygen or other gases.

**Oxygen deficiency** - A condition that occurs when a breathable atmosphere contains less than 19.5% oxygen. Note: normal air contains 20.8% oxygen.

**Physical hazard** - A chemical for which there is scientifically valid evidence that it is a combustible liquid, a compressed gas, an explosive, a flammable, an organic peroxide, an oxidizer, a pyrophoric, an unstable (reactive) or a water-reactive.

**Pyrophoric gases** - Gases that may spontaneously ignite in air at or below 54°C (130°F). Specific gases may not ignite in all circumstances or may explosively decompose.

**Restrictive Flow Orifice (RFO)** — A safety device placed in the outlet of a cylinder valve that is intended to limit the release rate of a hazardous gas to a maximum specified range in the event of the inadvertent opening of the valve, or failure of the system downstream of the valve outlet

**STP** – In Chemistry, Standard Temperature and Pressure or STP is defined as 0°C (32°F) and 1 atmosphere of pressure (101.325 kPa or 29.92 inches of Mercury).

**Toxic gas** – A gas that is poisonous or capable of causing injury or death, especially by chemical means.

**UEL** - Upper Explosive Limit - The highest concentration of a gas or vapor in air that can produce ignition or explosion.

**Unstable (Reactive)** – An unstable or reactive chemical can go through vigorous polymerization, decomposition or condensation. This process occurs when the chemical undergoes shock or changes in pressure or temperature.

**Water reactive** - A chemical that reacts with water to release a gas that is either flammable or presents a health hazard.

## **Appendix B**

### **Dewar Safety**

Dewars usually have Nitrogen as its common content. Contact of liquid nitrogen or any very cold gas with the skin or eyes may cause serious freezing (frostbite) injury. Protect hands at all times when working with liquid nitrogen.

### **Handle Liquid Nitrogen Carefully**

The extremely low temperature can freeze human flesh very rapidly. When spilled on a surface the liquid tends to cover it completely and intimately, cooling a large area. The gas issuing from the liquid is also extremely cold. Delicate tissue, such as that of the eyes, can be damaged by an exposure to the cold gas which would be too brief to affect the skin of the hands or face.

#### **Never allow any unprotected skin to touch objects cooled by liquid nitrogen.**

Such objects may stick fast to the skin and tear the flesh when you attempt to free yourself. Use tongs, preferable with insulated handles, to withdraw objects immersed in the liquid, and handle the object carefully.

### **Protective Clothing**

Protect your eyes with face shield or safety goggles (safety glasses without side shields do not give adequate protection). Always wear cryogenic gloves when handling anything that is, or may have been, in immediate contact with liquid nitrogen. The gloves should fit loosely, so that they can be thrown off quickly if liquid should splash into them. When handling liquid in open containers, it is advisable to wear high-top shoes. Trousers (which should be cuffless if possible) should be worn outside the shoes.

Any kind of canvas shoes should be avoided because a liquid nitrogen spill can be taken up by the canvas resulting in a far more severe burn, in fact that would occur if the feet were essentially open or bare! Now we don't advocate sandals when using liquid nitrogen, but we also don't think that the wearing of canvas shoes is a safe practice either.

**Approved Containers for low-temperature Liquids** Cryogenic containers are specifically designed and made of materials that can withstand the rapid changes and extreme temperature differences encountered in working with liquid nitrogen. Even these special containers should be filled *slowly* to minimize the internal stresses that occur when any material is cooled. Excessive internal stresses can damage the container.

Do not ever cover or plug the entrance opening of any liquid nitrogen dewar. Do not use any stopper or other device that would interfere with venting of gas.

These cryogenic liquid containers are generally designed to operate with little or no internal pressure. Inadequate venting can result in excessive gas pressure which could damage or burst the container. Use only the loose-fitting necktube core supplied or one of the approved accessories for closing the necktube. Check the unit periodically to be sure that venting is not restricted by accumulated ice or frost.

## **Proper Transfer Equipment**

Use a phase separator or special filling funnel to prevent splashing and spilling when transferring liquid nitrogen into or from a dewar. The top of the funnel should be partly covered to reduce splashing. Use only small, easily handled dewars for pouring liquid. For the larger, heavier containers, use a cryogenic liquid withdrawal device to transfer liquid from one container to another. Be sure to follow instructions supplied with the withdrawal device. When liquid cylinders or other large storage containers are used for filling, follow the instructions supplied with those units and their accessories.

## **Avoid Overfilling Containers**

Filling above the bottom of the necktube (or specified maximum level) can result in overflow and spillage of liquid when the necktube core or cover is placed in the opening.

## **Never use hollow rods or tubes as dipsticks**

When a warm tube is inserted into liquid nitrogen, liquid will spout from the bottom of the tube due to gasification and rapid expansion of liquid inside the tube. Wooden or solid metal dipsticks are recommended; avoid using plastics that may become very brittle at cryogenic temperatures which then become prone to shatter like a fragile piece of glass.

## **Nitrogen gas can cause suffocation without warning. Store and use liquid nitrogen only in a well ventilated place.**

- As the liquid evaporates, the resulting gas tends to displace the normal air from the area. In closed areas, excessive amounts of nitrogen gas reduces the concentration of oxygen and can result in asphyxiation. Because nitrogen gas is colorless, odorless and tasteless, it cannot be detected by the human senses and will be breathed as if it were air. Breathing an atmosphere that contains less than 18 percent oxygen can cause dizziness and quickly result in unconsciousness and death.

### **Note:**

The cloudy vapor that appears when liquid nitrogen is exposed to the air is condensed moisture, not the gas itself. The gas actually causing the condensation and freezing is completely invisible.

Never dispose of liquid nitrogen in confined areas or places where others may enter.

Disposal of liquid nitrogen should be done outdoors in a safe place. Pour the liquid slowly on gravel or bare earth where it can evaporate without causing damage. Do not pour the liquid on the pavement.



## **First Aid**

If a person seems to become dizzy or loses consciousness while working with liquid nitrogen, move to a well-ventilated area immediately. If breathing has stopped, apply artificial respiration. If breathing is difficult, give oxygen. Call in for an emergency. Keep warm and at rest.

If exposed to liquid or cold gas, restore tissue to normal body temperature 98.6° F (37° C) as rapidly as possible, followed by protection of the injured tissue from further damage and infection. Remove or loosen clothing that may constrict blood circulation to the frozen area. Call a physician. Rapid warming of the affected part is best achieved by using water at 108° F/42° C). Under no circumstances should the water be over 112° F/44° C, nor should the frozen part be rubbed either before or after rewarming. The patient should neither smoke, nor drink alcohol.

Most liquid nitrogen burns are really bad cases of frostbite. We don't mean to belittle the harm that can come from frostbite, but at the same time, we wanted to keep the dangers associated with liquid nitrogen burns in perspective. Indeed, liquid nitrogen burns could be treated as frostbite.

**Handling Liquid Nitrogen Dewars** Keep unit upright at all times except when pouring liquid from Dewars specifically designed for that purpose.

Tipping the container or laying it on its side can cause spillage of liquid nitrogen. It may also damage the container and any materials stored in it.

Rough handling can cause serious damage to dewars and refrigerators.

Dropping the container, allowing it to fall over on its side, or subjecting it to sharp impact or severe vibration can result in partial or complete loss of vacuum. To protect the vacuum insulation system, handle containers carefully. Do not "walk", roll or drag these units across a floor. Use a dolly or handcart when moving containers, especially the larger portable refrigerators. Large units are heavy enough to cause personal injury or damage to equipment if proper lifting and handling techniques are not used.

When transporting contents from a liquid nitrogen dewar, maintain adequate ventilation and protect the unit from damage.

Do not place these units in closed vehicles where the nitrogen gas that is continuously vented from unit can accumulate. Prevent spillage of liquids and damage to unit by securing it in the upright position so that it cannot be tipped over. Protect the unit from sever jolting and impact that could cause damage, especially to the vacuum seal.

## **Keep the unit clean and dry**

Do not store it in wet, dirty areas. Moisture, animal waste, chemicals, strong cleaning agents and other substances which could promote corrosion should be removed promptly. Use water or mild detergent for cleaning and dry the surface thoroughly. Do not use strong alkaline or acid cleaners that could damage the finish and corrode the metal shell.



**ARIZONA STATE UNIVERSITY**  
**Department of Environmental Health & Safety**  
Hazard Communication Program  
(Employee Right-to-Know)  
Pursuant to 29 CFR § 1910.1052

**Revision Date: Feb. 2009**

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## **Introduction and Purpose**

Approximately 32 million workers are potentially exposed to one or more chemical hazards daily. There are an estimated 650,000 existing chemical products, and hundreds of new ones are being introduced annually into the work place.

As a result of recognizing the serious nature of safety and health problems associated with worker exposure to hazardous chemicals and employer/employee lack of chemical knowledge, the Occupational Safety and Health Administration (OSHA) issued a final rule in 1983 called Hazard Communication, 29 CFR § 1910.1200, also known as Employee Right-To-Know. This rule applied to the employers in the manufacturing sector of industry. The scope of the rule was expanded in 1987 to include employers in the non-manufacturing sector. The primary goal of the standard is to assure employees and employers are adequately informed of chemical hazards in the work place and are provided with information on how to protect them while using hazardous chemicals.

It is the responsibility of ASU to provide a safe workplace for its employees. The main objective of the Arizona State University (ASU) Hazard Communications Program (Hazcom) is to minimize employee exposure to hazardous chemicals in the workplace. The HAZCOM ensures that employees are informed of the potential hazards in their workplace, and also the appropriate means to protect themselves. When chemicals are used by ASU employees in the performance of their duties, these activities shall be conducted in accordance with the provisions of the HAZCOM. The written HAZCOM shall be readily available to all employees, employee representatives and appropriate regulatory agencies upon request.

## **Scope and Application**

ASU is classified as a non-manufacturing employer where employees use a variety of hazardous chemicals (in smaller quantities compared to industry) during their employment. Therefore, the Hazard Communication Standard ("Employee Right-To-Know") applies to any ASU facility or department that uses hazardous chemicals. **Hazardous Chemicals** are defined by OSHA as any chemical which poses a physical hazard or a health hazard. This is determined by information in the MSDS. At ASU, we address the requirements of these regulations through this written Hazard Communication Program and our Laboratory Safety Program. Any use of Hazardous Chemicals other than in a laboratory registered with EH&S is covered by this program. Laboratory uses of Hazardous Chemicals are addressed through EHS 104 Hazardous Laboratory Chemicals at <http://www.asu.edu/aad/manuals/ehs/ehs104.html>. This exemption to this program and other are described below.

The ASU Hazard Communication Program covers all use of hazardous materials on campus except the following:

- Laboratory Reagents (for procedures applicable to laboratories, refer to the ASU Chemical Hygiene Program available at [http://www.asu.edu/uagc/EHS/documents/asu\\_chp.pdf](http://www.asu.edu/uagc/EHS/documents/asu_chp.pdf))

- Hazardous waste (for procedures applicable to hazardous waste, refer to the ASU Hazardous Waste Management Compliance Guidelines available at [http://uabf.asu.edu/ehs\\_hazmat\\_compliance\\_guidelines](http://uabf.asu.edu/ehs_hazmat_compliance_guidelines));
- Biological Hazards (for procedures applicable to biological hazards, refer to the ASU Biosafety Program available at [http://uabf.asu.edu/biosafety\\_program](http://uabf.asu.edu/biosafety_program));
- Ionizing and no ionizing radiation (for procedures applicable to radiation safety please refer to , the Office of Radiation Safety at <http://www.asu.edu/radiationsafety/>);
- Asbestos (for procedures applicable to asbestos management, refer to the ASU Asbestos Management Program available at [http://uabf.asu.edu/asbestos\\_management\\_program](http://uabf.asu.edu/asbestos_management_program) );
- Tobacco or tobacco products;
- Wood or wood products;
- Foods, drugs, or cosmetics intended for personal consumption by employees while in the workplace;
- Pesticides; or
- Work operations where employees only handle substances in sealed containers that are not opened.

The standard requires ASU to:

- Ensure hazard identification;
- Determine employee exposure to hazardous chemicals;
- Develop a written hazard communication Program;
- Inform employees of identified potential hazards;
- Provide training and information on safe work practices;
- Establish a file of the chemicals used;
- Acquire and distribute Material Safety Data Sheets (MSDS) for each chemical used;
- Maintain a container labeling system; and
- Establish record keeping procedures.

## **Responsibilities**

### Environmental Health & Safety

The Department of Environmental Health & Safety (EH&S) is charged with the overall responsibility to develop and implement a Hazard Communication Program for ASU. HAZCOM ensures regulatory compliance and provides employees with the information and training needed to protect them while using hazardous chemicals. EH&S is responsible for the following:

- Supporting Departmental Safety Committees as requested;
- Maintaining master chemical inventories for each campus;
- Providing technical assistance and training materials to departments;
- Maintaining training records;
- Serving as liaison to regulatory agencies; and

- Conducting audits and evaluations of program effectiveness.

#### Deans, Directors and Chairs

The Dean, Director or Chair for each department and/or college or their designees are responsible for the following:

- Ensuring participation of affected employees in EH&S required training programs;
- Establishing and implementing department specific information and training programs as recommended by EH&S;
- Maintaining required MSDS and ensuring availability to employees; and
- Providing to EH&S an inventory or Master List of hazardous chemicals used within their organizations;
- Notifying all vendors and contractors working in locations under their control of hazard chemicals that are in the area, making MSDS available to them and obtaining written acknowledgment of receipt of this information;
- Ensuring that prior to the initiation of work, any outside contractor or vendor provides the area department and/or unit with an MSDS for each chemical being used during the work project and that a copy of each MSDS is available at the location for each chemical being used entire duration the chemical is in use; and
- Utilizing the Sunrise system for all hazardous chemical purchases...
- Department; and
- HAZCOM.

#### Department Safety Committee Members

Each ASU employee is responsible for the following:

- Know the location and use the information provided in the MSDS;
- Ensure proper labeling of hazardous chemicals;
- Attend initial and follow-up hazard communication training as required;
- Report potential hazards, accidents and near-misses to supervisor immediately; and
- Assist supervisor in implementing recommendations for improving safety; and

#### Vendors, Contractors and Visitors

Each Vendor, Contractor and/or Visitor is responsible for the following:

- Notifying their ASU contact of their activities and reviewing any information provided related to Hazardous Chemicals in use at ASU;
- Providing MSDS and related hazard information to their ASU contact prior to utilizing any Hazard Chemical associated with their activities at ASU;
- Complying with all applicable EH&S regulations and ASU Policies related to their activities related to their purchase order, contract or any other agreement with ASU; and

- Informing each ASU department of any hazardous chemical(s) they may be using during the performance of their work.

### **Identification of Hazardous Materials**

The responsibility for determining whether a chemical is hazardous lies with the chemical manufacturer or importer of a chemical. End-users and/or supervisors may rely on the evaluation received from these suppliers, in the form of MSDSs and warning labels. A chemical inventory shall include a list of chemicals, including compressed gas cylinders, used in the workplace covered by HAZCOM and can be prepared by documenting the names of chemicals that have a warning label indicating a potential hazard (e.g., 4 flammable or corrosive). In addition to chemicals in containers, other substances generated in work operations such as welding fumes and some dusts shall also be listed in the inventory.

All identified chemicals must have a corresponding MSDS available in a binder or electronically. The binder or electronic file must be identified with the acronym MSDS on the spine or file name or folder and be located in an area accessible to all employees at all times, regardless of work shift.

### **Inventory of Hazardous Materials**

Departments that employ individuals who may be exposed to hazardous chemicals in the course of their job duties shall prepare a chemical inventory. The designated department shall maintain a current chemical inventory. A copy of the prepared chemical inventory shall be forwarded to Environmental Health & Safety at and updated at least annually. Environmental Health & Safety shall compile and maintain the Master Chemical Inventory for ASU. An example hazardous chemical inventory form can be found at

[http://www.asu.edu/uagc/EHS/forms/asu\\_chemical\\_inventory-template.xls](http://www.asu.edu/uagc/EHS/forms/asu_chemical_inventory-template.xls)

Chemical inventories shall be placed with a copy of the written Hazard Communication Program and stored in the Material Safety Data Sheet binder(s). This information shall be accessible to all employees at all times. A copy of the chemical inventory is to be forwarded to EH&S and updated at least annually.

The following list identifies, but is not limited to, some types of potentially hazardous chemical that may be present in the workplace:

Acids  
Adhesives  
Aerosols  
Battery Fluids  
Bleach  
Catalysts  
Caustics  
Cleaning Agents  
Coatings  
Compresses Gases

Degreasing Agents  
Dusts  
Etching Agents  
Flammables  
Foaming Resins  
Fungicides  
Gasoline  
Glues  
Greases  
Herbicides  
Industrial Oils  
Inks  
Insecticides  
Janitorial Supplies  
Kerosene  
Lacquers  
Lye  
Paints  
Pesticides  
Plastics  
Process Chemicals  
Resins  
Sealers  
Shellacs  
Solders  
Solvents  
Surfactants  
Thinners  
Varnishes  
Water Treatment Chemicals 5



## **Department Labels and Other Warnings**

Department supervisors are responsible for identifying hazardous chemicals in the workplace and effectively communicating information available from the manufacturers MSDS and labels or other cautionary warnings to employees.

All hazardous chemical containers in the work place must clearly identify, in English, the hazardous contents of the container. The supervisor has the responsibility to ensure that all employees under their supervision are aware of the requirements to have all hazardous chemical container labels affixed, legible and to contain the appropriate information and to enforce this requirement.

All secondary container(s) shall use either the NFPA or the Hazardous Materials Identification System (HMIS) label or manufacturers label of the appropriate size for the container. Supervisors will ensure that appropriate labels are available. If a manufacturer's label is unavailable, the appropriate information should be copied from the MSDS to the blank HMIS label (refer to Appendix C). If it is not practical to label a container, the proper chemical hazard information may be placed on a sign near the container, which is clearly visible to employees. Containers of hazardous chemicals at ASU must be received with a label that provides the appropriate identification and the hazards associated with the chemical. The label is to be supplied by the manufacturer, importer or distributor of the chemical. If the container arrives without a label, an HMIS label will be affixed to the container as outlined:

- Identity of chemicals (chemical or common name on the Material Safety Data Sheet);
- Name and address of the chemical manufacturer or distributor; and
- Appropriate hazard warning (designated by the chemical manufacturer or distributor).

Labels will not be removed unless the container is immediately re-labeled or the chemical in the container is emptied, cleaned and/or a new type of chemical is placed in the container, and the chemical container is re-labeled with the identity of the new chemical.

The HMIS labeling system operates on the same principle as the NFPA diamond. Blue indicates health hazard, red indicates flammability, yellow indicates reactivity, and special information (such as what personal protective equipment to wear) will be provided in the white section. It also uses a numerical system from 0-4 to indicate the severity of the hazard. Refer to the chart in Appendix C as a reference.

It should be noted that while the NFPA 704 Labeling system and the HMIS labeling system are similar but are not the same. Laboratories registered with EH&S is posted with an NFPA 704 label (referred to as an NFPA Diamond) as are chemical storage containers and chemical storage areas as required by local fire codes. When transferring information from the MSDS make certain which rating is being provide, because many manufacturers may use the NFPA rating or both ratings.

## **Material Safety Data Sheets**

Chemical manufacturers and distributors are required by OSHA to provide Material Safety Data Sheets (MSDS) to consumers. A MSDS is provided to ensure the end-user of chemical products is informed of the hazards associated with the use of the chemical and what safety precautions should be utilized. The same MSDS may be used for several chemicals if they have similar hazards and contents. Updated or new MSDSs will be distributed immediately upon receipt. Each department must maintain a complete and accurate MSDS for each chemical used in the workplace upon purchase of a chemical. When new and significant information becomes available concerning the hazards of a chemical or improved method of protection for employees, the chemical manufacturers, importers, or distributors must provide a MSDS with the updated information with the next shipment or within three months to the end-users.

If the manufacturer, importer or distributor fails to send a MSDS with a shipment labeled as a hazardous chemical, the department must obtain one from the chemical manufacturer, importer or distributor as soon as possible. Similarly, if the MSDS is incomplete or unclear, the department should contact the manufacturer, importer or distributor to get clarification or obtain the missing information (see page 10 for sample letters requesting a MSDS, or additional information for a MSDS). No chemical shall be used by any ASU employee unless a current MSDS is available.

MSDSs will usually be made up of at least ten (10) sections if they comply with the **Globally Harmonized System of Classification and Labeling of Chemicals** or **GHS** which is an internationally agreed upon system set to replace the various different classification and labeling standards used in different countries. The GHS will use consistent criteria for classification and labeling on a global level or the voluntary American National Standards Institute (ANSI) standard Z400.1, with each section describing a specific detail about the product. There may be up to 6 additional sections if information about a product for these sections is available. (Note: currently there is no mandated standard format for an MSDS; the format may vary depending on the manufacturer, importer, or distributor):

- Chemical product and company identification;
- Composition and/or information on hazardous ingredients;
- Hazards identification, including emergency overview;
- First aid measures;
- Fire-fighting measures;
- Accidental release measures;
- Handling and storage;
- Exposure controls and personal protection;
- Physical and chemical properties; and
- Stability and reactivity.

Additional sections may include information on toxicological, ecological, transport, disposal and regulatory information as well as any other information not covered in the above-mentioned sections.

A MSDS binder will be located in designated work areas. It is recommended that a brightly colored (red) binder with the acronym MSDS on the spine be used. All employees shall be informed of the location of the binder. In addition, the binder will be readily available to all employees at all times regardless of their work shift. The Department will forward new or updated MSDSs to the area Supervisor. The Coordinator will request additional information if the MSDS is unclear. The supervisor will maintain the MSDS in alphabetical order. If the MSDS replaces an older edition, the replacement will be kept in the back of the binder or forward it to the Department Supervisor to file in an archive MSDS binder.

Copies of the MSDS will be made available for any designated representative of the employee, or OSHA officer upon their request. The department and/or supervisor will be notified if a non-employee requests a copy of the MSDS.

### **Maintenance of Material Safety Data Sheets**

Each department will maintain a department MSDS master chemical file. Upon receipt of a new MSDS, the Department will update the master file. The Department will assist in performing an annual MSDS audit to ensure MSDSs for all chemicals listed in the chemical inventory are available in the workplace.

Supervisors will maintain the MSDS file and ensure the MSDS file is available for all employees to review at any time, all new MSDS received are filed, and that employees are aware of any new chemical introduced to the workplace. Supervisors will notify the Department if a listed chemical is no longer used or stored and will archive the MSDS from the file and update the chemical inventory.

### **Resources for Obtaining MSDSs**

MSDSs can be obtained by contacting the vendor selling the product or the product manufacturer. An example of a letter that can be sent to a manufacturer to request an MSDS follows. Most MSDS can be found by a simple web search such as goggle. If you have difficulty locating an MSDS please contact EH&S at [EHS@asu.edu](mailto:EHS@asu.edu) or (480) 965-6219.

### **Sample Letter Requesting a MSDS**

Blitz Manufacturing Company 1923 Oak Grove Lane Springfield, Massachusetts 02110

Dear Sir/Madam:

The Occupational Safety and Health Administration (OSHA) Hazard Communication Standard (29 CFR1910.1200) requires employers be provided Material Safety Data Sheets (MSDS) for all hazardous substances used in their facility, and to make these MSDS available to employees potentially exposed to these hazardous substances.

Therefore, we request a copy of the MSDS for your chemical product listed as (give product and/or chemical name here). We did not receive an MSDS with the initial shipment of (Blitz Solvent 90) we received on (date). We also request any additional information, supplemental

MSDS, or any other relevant data your company or supplier has concerning the safety and health aspects of this chemical product.

Please consider this letter a standing request to your company for any information concerning the safety and health aspects of using the chemical product that may be learned in the future. Delays in receiving the MSDS information will prevent use of your chemical product in our department. Please send the requested information to John Smith, Department, XYZ Department, Arizona State University, Tempe, Arizona 85287.

Please be advised, if we do not receive the MSDS for the above chemical by (date), we may have to notify OSHA of our inability to obtain this information. Your cooperation will be appreciated. Thank you for your timely response to this request.

Sincerely,

John Smith Department

If an MSDS does not contain the information required a follow up letter should be sent to the chemical manufacturer. An example of a follow up letter follows.

#### Sample Letter Requesting Additional MSDS Information

ACE Chemical Company, Incorporated 214 Capitol Drive Richmond, Virginia 23230

Dear Mr. Winston:

In an effort to comply with the Occupational Safety and Health Administration's Hazard Communication Standard, my department is seeking additional information on a chemical product manufactured by your company. The MSDS forwarded to our department appear to be deficient in the following areas:

1. Clear-VU 210 - no health effects listed
2. Clean-up 34 (Solvent) - no physical hazard listed

In order to provide adequate training for our employees and comply with the Standard, we must have completed MSDS, particularly with reference to the above-identified items. Please send the information to John Smith, Department, XYZ Department, Arizona State University, Tempe, Arizona 85287.

Thank you for your assistance in this matter.

Sincerely,

John Smith Department 10

#### Employee Information and Training

Departments shall have a written training and information program for all affected employees or ensure participation of affected employees in required EH&S training programs. Employee training shall be provided when employees are initially hired and when a new chemical hazard is introduced into the workplace. The workplace supervisor will ensure that employees are trained in the specific topics covered in the HAZCOM and provide further training relative to the specific hazardous chemicals employees will use in the performance of their duties.

EH&S provides training sessions at regular intervals during each semester through Human Resources Staff Development. Additionally, EH&S provides specific training for individual departments or principal investigators upon request.

At a minimum, employees shall be informed of:

- Requirements of OSHA's Hazard Communication Standard;
- The physical and health hazards of chemicals used in their workplace;
- Methods and observations that may be used to detect the presence or release of a hazardous chemical in the workplace;
- How to adequately protect themselves to minimize their exposure;
- Location of the chemical inventory and MSDS binder in the workplace;
- Details of ASU's HAZCOM, including an explanation of the labeling system and the MSDS and how employees can obtain and use the appropriate hazard information; and
- Location and availability of the written HAZCOM.

### **Trade Secrets**

A trade secret allows a manufacturer to maintain their market advantage over competitors by designating certain chemical formulations as being proprietary. For example, a trade secret may be a confidential device, pattern, information or chemical formulation. Chemical industry trade secrets are generally formulas, process data, or a "specific chemical identity." The latter is the type of trade secret information referred to in the Hazard Communication Standard. The term "a trade secret" includes the chemical name, the Chemical Abstracts Services (CAS) Registry Number, or any other specific information that reveals the chemical's precise designation. It does not include common names.

The standard maintains a balance between the need to protect exposed employees and the employer's need to maintain confidentiality of a legitimate trade secret. This is achieved by providing for limited disclosure to health professionals who are providing medical or other occupational health services to exposed employees, employees and their designated representatives, under specified conditions of need and confidentiality.

The chemical manufacturer, importer, or employer must immediately disclose the specific chemical identity of the hazardous chemical to a treating physician or nurse when the information is needed for proper emergency or first-aid treatment. As soon as circumstances permit, the chemical manufacturer, importer, or employer may obtain a written statement of need and a confidentiality agreement.

Under the contingency described here, the treating physician or nurse has the ultimate responsibility for determining that a medical emergency exists. At the time of the emergency, the professional judgment of the physician or nurse regarding the situation must form the basis for triggering the immediate disclosure requirement. Because the chemical manufacturer, importer, or employer can demand a written statement of need and a confidentiality agreement to be completed after the emergency is abated, further disclosure of the trade secret can be effectively controlled.

In non-emergency situations, chemical manufacturers, importers, or employers must disclose the withheld specific chemical identity to health professionals providing medical or other occupational health services

to exposed employees, and to employees and their designated representatives, if certain conditions are met.

In this context, "health professionals" include physicians, occupational health nurses, industrial hygienists, toxicologists, or epidemiologist.

The request for information must be in writing and must describe with reasonable detail the medical or occupational health need for the information. The request will be considered if the information will be used for one or more of the following activities:

- Assess the hazards of the chemicals to which employees will be exposed;
- Conduct or assess sampling of the work place atmosphere to determine employee exposure levels;
- Conduct pre-assignment or periodic medical surveillance of exposed employees;
- Provide medical treatment to exposed employees;
- Select or assess appropriate personal protective equipment for exposed employees;
- Design or assess engineering controls or other protective measures for exposed employees; and
- Conduct studies to determine the health effects of exposure.

Health professionals, employees, or their designated representatives must specify why alternative information is insufficient. The request for information must explain in detail why disclosure of the specific chemical identity is essential, and include the procedures to be used to protect the confidentiality of the information. It must not include an agreement not to use the information for any purpose other than the stated need, nor shall the information be release to unauthorized individuals and/or entities under any circumstances.

### **Emergency Procedures**

Each department must develop emergency procedures specific to their operation(s) and all affected employees must be aware of these procedures. This Program should include (but not limited to) actions or contingencies for:

- Evacuations due to fires, chemical spills, and other situations;
- First aid;
- Shut down, lock out during evacuations; and
- Location of emergency equipment (fire extinguishers, fire alarm pull stations, showers, eyewashes, etc.).

Protocol for handing emergencies is outlined in the ASU Emergency Response Guide available at [http://www.asu.edu/uagc/emergency/response\\_guide.html](http://www.asu.edu/uagc/emergency/response_guide.html).

Faculty, staff, and students who discover or are involved in a hazardous chemical emergency are responsible for taking appropriate action to protect themselves and the university community by notifying the appropriate authorities and following established protocol outlined in the ASU

Emergency Response Guide. The Emergency Response Guide must be available in areas that have the potential for chemical emergencies, and be posted in a conspicuous area. The Emergency Response Guide is available on the EH&S website at [http://uabf.asu.edu/ehs\\_services](http://uabf.asu.edu/ehs_services) or a copy can be obtained by calling EH&S at (480) 965-6219.

### **Hazard Communication and Outside Contractors**

#### **Notification Procedure**

Each department is responsible for notifying all vendors and contractors working in locations under their control of hazardous chemicals that are in the area, making MSDS available to them and obtaining written acknowledgment of receipt of this information. To accomplish this each department should provide the following information to vendors and contractors working in areas under their control:

- A list of all chemicals located in the work area where the outside contractor or vendor will be performing the work; • Provide MSDS(s) upon request to the contractor for all listed chemicals; and
- Obtain a written documentation of the contractor's or vendor's acknowledgement that the information was provided or made available prior to the initiation of the contractor's work.

Prior to the initiation of work, outside contractors are to provide the area department and/or unit with an MSDS for each chemical being used during the work project and shall maintain a copy for each chemical being used at the work site for the entire duration the chemical is in use.

Facilities Programming and Construction will:

- Forward the contractor MSDS(s) to the area supervisor and/or department and Environmental Health & Safety with information regarding which work area(s) will be affected;
- Establish a file for the contractor MSDS(s) and forward a copy to each area supervisor and/or department that may be affected by the project;
- Supervisors and/or department representatives shall review the contractor MSDS(s) with department and/or unit employees;
- Once the contractor completes the project, ensure contractors remove all project related chemicals from the work area; department representative are no longer kept on location; and
- Notify the department supervisor and/or department representative that the contractor MSDS(s) from the area file or location have been or may be removed.

## **Accident Reporting**

Employees shall report accidents and injuries to their supervisor immediately. Supervisors shall submit a report to Environmental Health & Safety for any accident, injury or near miss within 72 hours as identified in EHS 115: Incident Reporting and Investigation available at <http://www.asu.edu/aad/manuals/ehs/ehs115.html>. All employees will be free from any reprisals for reporting accidents. Accident reporting will assist Environmental Health & Safety in providing corrective procedures to avoid a recurrence of the accident.

## **Audits**

Departments, departments, managers and supervisors are responsible for establishing, implementing and maintaining a system of communication to relay health and safety information to employees. Resources available through ASU's EH&S department should be used whenever available but other sources may be used if approved through EH&S.

Supervisors and safety committees may conduct regular, periodic audits of the workplace to evaluate work practices and identify potential hazards. The frequency of audits should be determined by the level of risk associated with a department's specific operation or process. Audits should be conducted annually or whenever there is a change in the type of hazardous chemicals, processes, procedures, or equipment used which may alter the hazards posed to employees. The department, safety committee and supervisors should evaluate the effectiveness of the HAZCOM program for their specific work area. For more information or training associated with conducting audits or other assessments under this program, please contact EH&S at [EHS@asu.edu](mailto:EHS@asu.edu).

Environmental Health & Safety will conduct periodic inspections, as necessary, determining individual department compliance with HAZCOM. Inspections may be performed in conjunction with the department's periodic audit by the department representative and/or safety committee members or compliance officers. Inspection reports will document opportunities for improvement and be directed to the Department Dean, Director, Chair or designee.

Corrective action is to be completed in a reasonable amount of time and documented. Improvement opportunities related to training, labeling or maintenance of MSDS should be completed within 30 days of the finding.

Audit reports shall include the date the audit was conducted and the name of the employee(s) conducting the audit.

## **Program Evaluation**

Periodic program evaluations will be conducted by EH&S. Program evaluations will assess the effectiveness of the systems in place to implement the requirements of this program.



## **Record Keeping**

Documentation and records associated with this program shall be maintained as required by the Hazard Communication Standard, 29 CFR § 1910.1200. Environmental Health & Safety, individual departments, supervisors, and other department representatives shall maintain records as indicated below.

- Each department shall maintain the most recent Chemical Inventories associated with their operations
- Each department shall maintain MSDS including archives of hazardous chemicals no longer in use.
- EH&S shall maintain copies of employee training records for any training program presented by EH&S.
- Departments who present their own training under this program shall forward copies of training records to EH&S for long term maintenance.

Records shall be made available to employees and/or their representatives upon request.

# **Appendix A**

## **Definitions**

**ACGIH:** American Conference of Governmental Industrial Hygienists; an organization of professional personnel in governmental agencies or educational institutions engaged in occupational safety and health programs. ACGIH develops and publishes recommended occupational exposure limits (see "TLV") for hundreds of chemical substances and physical agents.

**Acute:** Severe, often dangerous conditions in which relatively rapid changes occur.

**Acute Exposure:** A single, brief exposure to toxic substances. Effects (i.e., adverse effects on the human body) if any are evident soon after the exposure, could come quickly to a crisis.

**Alloys:** A mixture of metal (such as brass), in some cases a metal and a non-metal.

**Ambient Temperature:** Temperature of the immediate surroundings.

**Appearance/Odor:** The color, physical state at room temperature, size of particles, characteristics of the material. Odor is described in comparison to common familiar "smells." Threshold refers to the concentration required in the air before vapors are detected or recognized.

**Asphyxiant:** A chemical (gas or vapor) that can cause death or unconsciousness by suffocation. Simple asphyxiants such as nitrogen, either use up or displace oxygen in the air. They become especially dangerous in confined or enclosed spaces. Chemical asphyxiant, such as carbon monoxide and hydrogen sulfide, interfere with the body's ability to absorb or transport oxygen to the tissues.

**Aspiration Hazard:** The danger of drawing a fluid into the lungs and causing an inflammatory response to occur.

**Autoignition Temperature:** Lowest temperature at which a flammable gas or vapor-air mixture will ignite from its own heat source or other contacted heat source.

**Boiling Point:** Temperature at which vapor pressure of a liquid equals atmospheric pressure.

**C.A.S. Number:** The number assigned to chemicals or products by the Chemical Abstracts Service.

**Carcinogen:** A substance or agent capable of causing or producing cancer.

**Catalyst:** A substance which changes the speed of a chemical reaction but undergoes no permanent change itself. An example of a catalyst is the platinum used in automotive catalytic converters on the exhaust system.

**Chronic Effect:** An adverse effect on a human or animal. Symptoms develop slowly over a long period of time or recur frequently.

**Combustible:** A substance capable of fueling a fire. Also a term used to classify certain liquids on the basis of their flashpoints. Also see "flammable".

**Compressed Gas:** A gas under pressure which is greater than that of the atmosphere. An example is the air in automobile tires.

**Corrosive Material:** As defined by the Department of Transportation (DOT), a corrosive material is a liquid or solid that causes visible destruction or irreversible alterations in human skin tissue at the site of contact; or in the cases of leakage from its packaging, a liquid that has a severe corrosion rate on steel.

**Cutaneous:** Pertaining to or affecting the skin.

**Decomposition:** Breakdown of a material or substance (by heat, chemical reaction, electrolysis, decay or other processes) into simpler substances.

**Dermal:** Pertaining to or affecting the skin.

**Dyspnea:** Shortness of breath, difficult or labored breathing.

**Erythema:** A reddening of the skin. 16

**Evaporation Rate:** The ratio of time required to evaporate the same volume of a reference liquid (ether). The higher the ratio, the slower the evaporation rate.

**Explosive:** A chemical that causes a sudden release of pressure, gas and heat when subjected to shock, pressure, or high temperature.

**Exposure Limit:** Limit set to minimize occupational exposure to a hazardous substance. Recommended occupational exposure limits used are American Council of Governmental Industrial Hygienists' Threshold Limit Values (TLV) and Occupational Safety and Health Administration Permissible Exposure Limits (PEL).

**Extinguishing Agents (Methods):** Agent(s) suitable for controlling or putting out a fire, when properly applied.

**Flammable:** A material which is easily ignited and burns with extreme rapidity.

**Flammable Limits:** The range of a vapor/gas concentration in air that will burn or explode if an ignition source is present.

**Flash Point:** The minimum temperature at which a liquid gives off sufficient vapor to form, with air, an ignitable mixture.

**General Exhaust:** Removal of contaminated air from a large area by an air circulation or exchange system.

**Generic Substance:** A substance identified by its general chemical name and/or formula.

**Hazard Communication Program (HAZCOM):** The written program employers must develop and use which specifies employee training for routine and emergency use of all potentially hazardous chemicals in the work place, details pertaining to chemical labels, storage and Material Safety Data Sheets and a complete list of all hazardous chemicals in the work place.

**Hazardous Chemical:** Any chemical which poses a physical hazard or a health hazard. This is determined by information in the MSDS.

**Health Hazard:** Any chemical for which there is at least one scientific study that shows it may cause acute or chronic health symptoms. This includes chemicals which are carcinogens, toxic or highly toxic, irritants, corrosives, sensitizers, or chemicals that effect target organs including the lungs, kidneys, nervous system, pulmonary system, reproductive system, skin and eyes.

**Highly Toxic:**

- A chemical which has been found through testing of laboratory animals to cause death when exposed at certain levels.
- A chemical is highly toxic to ingest if it has a median lethal dose (LD50) of less than 50 mg/kg. This means that 50 percent of the test animals (rats) died when given an oral dosage of 50 milligrams for each kilogram of body weight.
- A chemical is highly toxic to touch if it has an (LD50) rating of less than 200 mg/kg, meaning that 50 percent of the lab animals (rabbits) die after having continuous skin contact at that dosage for 24 hours or less.
- A chemical is highly toxic to breathe if it has a (LC50) rating of less than 200 PPM for gas or vapor and a 2 mg/L for dust, fume, or mist when exposed for an hour or less.

**Ignition Source:** Anything that provides heat, sparks, or flame sufficient to cause combustion/explosion.

**Incompatible:** Materials which could cause dangerous reactions from direct contact with one another are described as incompatible.

**Ingestion:** The drawing of a substance into the body (lungs) through the nose, mouth, and breathing passages, in the form of a gas, vapor, fume, mist, or dust. 17

**Irritant:** A substance which will cause an inflammatory response or reaction of the eye, skin, or respiratory system, following single or multiple exposures.

**LC50:** Lethal Concentration 50; a single dose of material which on the basis of laboratory tests is expected to kill 50% of a group of test animals when administered by mouth (oral) or applied to the skin (dermal or cutaneous).

**LD50:** Lethal Dose 50; a single dose of material which on the basis of laboratory tests is expected to kill 50% of a group of test animals. The material may be administered by mouth (oral) or applied to the skin (dermal or cutaneous).

**LEL (Lower Explosive Limit):** The lowest concentration of a gas or vapor in the air that can produce ignition or explosion.

**Local Exhaust:** A system for capturing and exhausting contaminants from the air at the point where the contaminants (gases, particulates) are released. Not to be confused with "general exhaust".

**MSDS (Material Safety Data Sheet):** Written or printed material about a chemical that specifies its hazards, safe use and other information. It is prepared by the chemical manufacturer, and is required by federal law.

**Mechanical Exhaust:** A powered device, such as a motor-driven fan or air/street venturi tube, for exhausting contaminants from a work place, vessel, or enclosure.

**Narcosis:** Stupor or unconsciousness caused by exposure to a chemical.

**Neutralize:** To render chemically neutral or harmless; neither acid nor base; to counteract the activity or effect of; the addition of a base (sodium hydroxide) to an acid (hydrochloric acid) results in water and a salt (sodium chloride), thus the acid has been "neutralized" or rendered harmless.

**Odor Threshold:** The minimum concentration of an airborne, toxic substance whose odor is detectable to the average individual. Depending on whether it is above or below substances TLV, it may be indicative of whether additional ventilation is required.

**Oral:** Of, through, pertaining to, or affecting the mouth.

**OSHA:** Occupational Safety and Health Administration of the U.S. Department of Labor; a federal agency with safety and health enforcement authority for most of U.S. industry and business.

**Oxidizer:** Department of Transportation defines oxidizer or oxidizing material as a substance that yields oxygen readily to stimulate the combustion (oxidation) of organic matter. Chlorate ( $\text{ClO}_3$ ), permanganate ( $\text{MnO}_4$ ) and nitrate ( $\text{NO}_3$ ) compounds are examples of oxidizers.

**PEL (Permissible Exposure Limit):** An exposure limit established by OSHA's regulatory authority. May be a time weighted average (TWA) limit or a maximum concentration exposure limit.

**Personal Protective Equipment (PPE):** Equipment designed to protect worker health and safety, e.g., chemical resistant gloves, safety glasses or goggles, face shields, etc.

**PPM (parts per million):** The unit for measuring the concentration of a gas or vapor in contaminated air. Also used to indicate the concentration of a particular substance in a liquid or solid.

**Physical Hazard:** A chemical which is proved to be a combustible liquid, compressed gas, explosive, flammable, oxidizer, pyrophoric, unstable (reactive) or water-reactive.

**Polymerization:** A chemical reaction in which a large number of relatively simple molecules combine to form a large chainlike molecule. A hazardous polymerization is a reaction which takes place at a rate which releases large amounts of energy.

**Pyrophoric:** A chemical which ignites spontaneously with air at 130 degrees F. or less. 18

**Respiratory Protection:** Devices for use in conditions exceeding set exposure levels. When properly selected, maintained and worn by the user, it will protect the users' respiratory system from exposure to airborne contaminants by inhalation.

**SCBA:** Self-contained breathing apparatus.

**Sensitizer:** A substance, which on first exposure, causes little or no reaction in man or test animals, but which on subsequent exposure(s) may cause a marked response not necessarily limited to the contact site. Skin sensitization is the most common form of the problem in the industrial setting, although respiratory sensitization to a few chemicals has been known to occur.

**Solubility in Water:** The percentage of a material (by weight) that will dissolve in water at a specific temperature.

- NEGLIGIBLE LESS THAN 0.1%
- LIGHT 0.1 TO 1.0%
- MODERATE 1 TO 10%
- APPRECIABLE MORE THAN 10%
- COMPLETE SOLUBLE IN ALL PROPORTIONS

**Solvent:** A substance which dissolves another substance.

**Specific Gravity:** The ratio of weight of volume of material to the weight of an equal volume of water usually at 60 F., otherwise specified  $H_2O-1$ .

**Systemic:** Spread throughout the body, affecting many or all body systems or organs, not localized in one spot or area.

**TLV "Skin":** This designation sometimes appears alongside a TLV of PEL. It refers to the possibility of absorption of the particular chemical through the skin and eyes. Thus, the protection of large surface areas of skin should be considered to prevent skin absorption so that the TLV is not invalidated.

**Target Organ:** The specific organs or body systems that sustain hazardous effects from a toxic chemical are either long or short-term. Target organs could be the liver, kidney, central nervous system or skin.

**Toxic:** A substance which has a median lethal dose (LD50) of 50 to 500 mg/kg for ingestion, from 200 to 1,000 mg/kg within a 24-hour period for contact and from 200 to 2,000 PPM gas or vapor for inhalation.

**UEL (Upper Explosive Limit):** The highest concentration of a gas or vapor in air that can produce ignition or explosion.

**Unstable (Reactive):** A chemical which vigorously undergoes polymerization, decomposition, or condensation via shock, pressure, or temperature.

**Vapor Density:** The ratio of the density of a substance's vapor to the density of another substance's vapor, usually air. A vapor density of greater than one means that the substance is heavier than air.

**Vapor Pressure:** The pressure exerted by vapor, in confinement, over its liquid as it accumulates at a constant temperature.

**Water Reactive:** A chemical which reacts with water is to form flammable gas or produce a health hazard. 19



**Appendix B**  
**29 CFR § 1910.1200**  
**Hazard Communication**

• **Part Number:**

1910

• **Part Title:**

Occupational Safety and Health Standards

• **Subpart:**

Z

• **Subpart Title:**

Toxic and Hazardous Substances

• **Standard Number:**

1910.1200

• **Title:**

Hazard Communication.

• **Appendix:**

A, B, C, D, E

**1910.1200(a)**

"Purpose."

**1910.1200(a)(1)**

The purpose of this section is to ensure that the hazards of all chemicals produced or imported are evaluated, and that information concerning their hazards is transmitted to employers and employees. This transmittal of information is to be accomplished by means of comprehensive hazard communication programs, which are to include container labeling and other forms of warning, material safety data sheets and employee training.

**1910.1200(a)(2)**

**1910.1200(a)(2)**

This occupational safety and health standard is intended to address comprehensively the issue of evaluating the potential hazards of chemicals, and communicating information concerning hazards and appropriate protective measures to employees, and to preempt any legal requirements of a state, or political subdivision of a state, pertaining to this subject. Evaluating the potential hazards of chemicals, and communicating information concerning hazards and appropriate protective measures to employees, may include, for example, but is not limited to, provisions for: developing and maintaining a written hazard communication program for the workplace, including lists of hazardous chemicals present; labeling of containers of chemicals in the workplace, as well as of containers of chemicals being shipped to other workplaces; preparation and distribution of material safety data sheets to employees and downstream employers; and development and implementation of employee training programs regarding hazards of chemicals and protective measures. Under section 18 of the Act, no state or political subdivision of a state may adopt or enforce, through any court or agency, any requirement relating to the issue addressed by this Federal standard, except pursuant to a Federally-approved state program.

**1910.1200(b)**

"Scope and application."

**1910.1200(b)(1)**

This section requires chemical manufacturers or importers to assess the hazards of chemicals which they produce or import, and all employers to provide information to their employees about the hazardous chemicals to which they are exposed, by means of a hazard communication

program, labels and other forms of warning, material safety data sheets, and information and training. In addition, this section requires distributors to transmit the required information to employers. (Employers who do not produce or import chemicals need only focus on those parts of this rule that deal with establishing a workplace program and communicating information to their workers. Appendix E of this section is a general guide for such employers to help them determine their compliance obligations under the rule.)

**1910.1200(b)(2)**

This section applies to any chemical which is known to be present in the workplace in such a manner that employees may be exposed under normal conditions of use or in a foreseeable emergency.

**1910.1200(b)(3)**

This section applies to laboratories only as follows:

**1910.1200(b)(3)(i)**

Employers shall ensure that labels on incoming containers of hazardous chemicals are not removed 21 or defaced;

**..1910.1200(b)(3)(ii)**

**1910.1200(b)(3)(ii)**

Employers shall maintain any material safety data sheets that are received with incoming shipments of hazardous chemicals, and ensure that they are readily accessible during each work shift to laboratory employees when they are in their work areas;

**1910.1200(b)(3)(iii)**

Employers shall ensure that laboratory employees are provided information and training in accordance with paragraph (h) of this section, except for the location and availability of the written hazard communication program under paragraph (h)(2)(iii) of this section; and,

**1910.1200(b)(3)(iv)**

Laboratory employers that ship hazardous chemicals are considered to be either a chemical manufacturer or a distributor under this rule, and thus must ensure that any containers of hazardous chemicals leaving the laboratory are labeled in accordance with paragraph (f)(1) of this section, and that a material safety data sheet is provided to distributors and other employers in accordance with paragraphs (g)(6) and (g)(7) of this section.

**1910.1200(b)(4)**

In work operations where employees only handle chemicals in sealed containers which are not opened under normal conditions of use (such as are found in marine cargo handling, warehousing, or retail sales), this section applies to these operations only as follows:

**1910.1200(b)(4)(i)**

Employers shall ensure that labels on incoming containers of hazardous chemicals are not removed or defaced;

**..1910.1200(b)(4)(ii)**

**1910.1200(b)(4)(ii)**

Employers shall maintain copies of any material safety data sheets that are received with incoming shipments of the sealed containers of hazardous chemicals, shall obtain a material safety data sheet as soon as possible for sealed containers of hazardous chemicals received without a material safety data sheet if an employee requests the material safety data sheet, and

shall ensure that the material safety data sheets are readily accessible during each work shift to employees when they are in their work area(s); and,

**1910.1200(b)(4)(iii)**

Employers shall ensure that employees are provided with information and training in accordance with paragraph (h) of this section (except for the location and availability of the written hazard communication program under paragraph (h)(2)(iii) of this section), to the extent necessary to protect them in the event of a spill or leak of a hazardous chemical from a sealed container.

**1910.1200(b)(5)**

This section does not require labeling of the following chemicals:

**1910.1200(b)(5)(i)**

Any pesticide as such term is defined in the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 136 et seq.), when subject to the labeling requirements of that Act and labeling regulations issued under that Act by the Environmental Protection Agency;

**1910.1200(b)(5)(ii)**

Any chemical substance or mixture as such terms are defined in the Toxic Substances Control Act (15 U.S.C. 2601 et seq.), when subject to the labeling requirements of that Act and labeling regulations issued under that Act by the Environmental Protection Agency;

**1910.1200(b)(5)(iii)**

**1910.1200(b)(5)(iii)**

Any food, food additive, color additive, drug, cosmetic, or medical or veterinary device or product, including materials intended for use as ingredients in such products (e.g. flavors and fragrances), as such terms are defined in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.) or the Virus-Serum-Toxin Act of 1913 (21 U.S.C. 151 et seq.), and regulations issued under those Acts, when they are subject to the labeling requirements under those Acts by either the Food and Drug Administration or the Department of Agriculture;

**1910.1200(b)(5)(iv)**

Any distilled spirits (beverage alcohols), wine, or malt beverage intended for nonindustrial use, as such terms are defined in the Federal Alcohol Administration Act (27 U.S.C. 201 et seq.) and regulations issued under that Act, when subject to the labeling requirements of that Act and labeling regulations issued under that Act by the Bureau of Alcohol, Tobacco, and Firearms;

**1910.1200(b)(5)(v)**

Any consumer product or hazardous substance as those terms are defined in the Consumer Product Safety Act (15 U.S.C. 2051 et seq.) and Federal Hazardous Substances Act (15 U.S.C. 1261 et seq.) respectively, when subject to a consumer product safety standard or labeling requirement of those Acts, or regulations issued under those Acts by the Consumer Product Safety Commission; and,

**1910.1200(b)(5)(vi)**

Agricultural or vegetable seed treated with pesticides and labeled in accordance with the Federal Seed Act (7 U.S.C. 1551 et seq.) and the labeling regulations issued under that Act by the Department of Agriculture.

**1910.1200(b)(6)**

**1910.1200(b)(6)**

This section does not apply to:

**1910.1200(b)(6)(i)**

Any hazardous waste as such term is defined by the Solid Waste Disposal Act, as amended by the Resource Conservation and Recovery Act of 1976, as amended (42 U.S.C. 6901 et seq.), when subject to regulations issued under that Act by the Environmental Protection Agency;

**1910.1200(b)(6)(ii)**

Any hazardous substance as such term is defined by the Comprehensive Environmental Response, Compensation and Liability ACT (CERCLA) (42 U.S.C. 9601 et seq.) when the hazardous substance is the focus of remedial or removal action being conducted under CERCLA in accordance with the Environmental Protection Agency regulations.

**1910.1200(b)(6)(iii)**

Tobacco or tobacco products;

**1910.1200(b)(6)(iv)**

Wood or wood products, including lumber which will not be processed, where the chemical manufacturer or importer can establish that the only hazard they pose to employees is the potential for flammability or combustibility (wood or wood products which have been treated with a hazardous chemical covered by this standard, and wood which may be subsequently sawed or cut, generating dust, are not exempted);

**1910.1200(b)(6)(v)**

Articles (as that term is defined in paragraph (c) of this section);

**1910.1200(b)(6)(vi)**

Food or alcoholic beverages which are sold, used, or prepared in a retail establishment (such as a grocery store, restaurant, or drinking place), and foods intended for personal consumption by employees while in the workplace;

**1910.1200(b)(6)(vii)**

**1910.1200(b)(6)(viii)**

Any drug, as that term is defined in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), when it is in solid, final form for direct administration to the patient (e.g., tablets or pills); drugs which are packaged by the chemical manufacturer for sale to consumers in a retail establishment (e.g., over-the-counter drugs); and drugs intended for personal consumption by employees while in the workplace (e.g., first aid supplies);

**1910.1200(b)(6)(ix)**

Cosmetics which are packaged for sale to consumers in a retail establishment, and cosmetics intended for personal consumption by employees while in the workplace; 22 23

**1910.1200(b)(6)(x)**

Any consumer product or hazardous substance, as those terms are defined in the Consumer Product Safety Act (15 U.S.C. 2051 et seq.) and Federal Hazardous Substances Act (15 U.S.C. 1261 et seq.) respectively, where the employer can show that it is used in the workplace for the purpose intended by the chemical manufacturer or importer of the product, and the use results in a duration and frequency of exposure which is not greater than the range of exposures that could reasonably be experienced by consumers when used for the purpose intended;

**1910.1200(b)(6)(xi)**

Nuisance particulates where the chemical manufacturer or importer can establish that they do not pose any physical or health hazard covered under this section;

**1910.1200(b)(6)(xii)**

Ionizing and nonionizing radiation; and,

**1910.1200(b)(6)(xii)**

Biological hazards.

**1910.1200(c)**

"Definitions."

"Article" means a manufactured item other than a fluid or particle: (i) which is formed to a specific shape or design during manufacture; (ii) which has end use function(s) dependent in whole or in part upon its shape or design during end use; and (iii) which under normal conditions of use does not release more than very small quantities, e.g., minute or trace amounts of a hazardous chemical (as determined under paragraph (d) of this section), and does not pose a physical hazard or health risk to employees.

"Assistant Secretary" means the Assistant Secretary of Labor for Occupational Safety and Health, U.S. Department of Labor, or designee.

"Chemical" means any element, chemical compound or mixture of elements and/or compounds.

"Chemical manufacturer" means an employer with a workplace where chemical(s) are produced for use or distribution.

"Chemical name" means the scientific designation of a chemical in accordance with the nomenclature system developed by the International Union of Pure and Applied Chemistry (IUPAC) or the Chemical Abstracts Service (CAS) rules of nomenclature, or a name which will clearly identify the chemical for the purpose of conducting a hazard evaluation.

"Combustible liquid" means any liquid having a flashpoint at or above 100 deg. F (37.8 deg. C), but below 200 deg. F (93.3 deg. C), except any mixture having components with flashpoints of 200 deg. F (93.3 deg. C), or higher, the total volume of which make up 99 percent or more of the total volume of the mixture.

"Commercial account" means an arrangement whereby a retail distributor sells hazardous chemicals to an employer, generally in large quantities over time and/or at costs that are below the regular retail price.

"Common name" means any designation or identification such as code name, code number, trade name, brand name or generic name used to identify a chemical other than by its chemical name.

"Compressed gas" means:

(i) A gas or mixture of gases having, in a container, an absolute pressure exceeding 40 psi at 70 deg. F (21.1 deg. C); or

(ii) A gas or mixture of gases having, in a container, an absolute pressure exceeding 104 psi at 130 deg. F (54.4 deg. C) regardless of the pressure at 70 deg. F (21.1 deg. C); or

(iii) A liquid having a vapor pressure exceeding 40 psi at 100 deg. F (37.8 deg. C) as determined by ASTM D-323-72.

"Container" means any bag, barrel, bottle, box, can, cylinder, drum, reaction vessel, storage tank, or the like that contains a hazardous chemical. For purposes of this section, pipes or piping systems, and engines, fuel tanks, or other operating systems in a vehicle, are not considered to be containers.

"Designated representative" means any individual or organization to which an employee gives written authorization to exercise such employee's rights under this section. A recognized or certified 24 collective bargaining agent shall be treated automatically as a designated representative without regard to written employee authorization.

"Director" means the Director, National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, or designee.

"Distributor" means a business, other than a chemical manufacturer or importer, which supplies hazardous chemicals to other distributors or to employers.

"Employee" means a worker who may be exposed to hazardous chemicals under normal operating conditions or in foreseeable emergencies. Workers such as office workers or bank tellers who encounter hazardous chemicals only in non-routine, isolated instances are not covered.

"Employer" means a person engaged in a business where chemicals are either used, distributed, or are produced for use or distribution, including a contractor or subcontractor.

"Explosive" means a chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.

"Exposure or exposed" means that an employee is subjected in the course of employment to a chemical that is a physical or health hazard, and includes potential (e.g. accidental or possible) exposure. "Subjected" in terms of health hazards includes any route of entry (e.g. inhalation, ingestion, skin contact or absorption.)

"Flammable" means a chemical that falls into one of the following categories:

(i) "Aerosol, flammable" means an aerosol that, when tested by the method described in 16 CFR 1500.45, yields a flame projection exceeding 18 inches at full valve opening, or a flashback (a flame extending back to the valve) at any degree of valve opening;

(ii) "Gas, flammable" means: (A) A gas that, at ambient temperature and pressure, forms a flammable mixture with air at a concentration of thirteen (13) percent by volume or less; or (B) A gas that, at ambient temperature and pressure, forms a range of flammable mixtures with air wider than twelve (12) percent by volume, regardless of the lower limit;

(iii) "Liquid, flammable" means any liquid having a flashpoint below 100 deg. F (37.8 deg. C), except any mixture having components with flashpoints of 100 deg. F (37.8 deg. C) or higher, the total of which make up 99 percent or more of the total volume of the mixture.

(iv) "Solid, flammable" means a solid, other than a blasting agent or explosive as defined in 1910.109(a), that is liable to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a serious hazard. A chemical shall be considered to be a flammable solid if, when tested by the method described in 16 CFR 1500.44, it ignites and burns with a self-sustained flame at a rate greater than one-tenth of an inch per second along its major axis.

"Flashpoint" means the minimum temperature at which a liquid gives off a vapor in sufficient concentration to ignite when tested as follows:

(i) Tagliabue Closed Tester (See American National Standard Method of Test for Flash Point by Tag Closed Tester, Z11.24-1979 (ASTM D 56-79)) for liquids with a viscosity of less than 45 Saybolt Universal Seconds (SUS) at 100 deg. F (37.8 deg. C), that do not contain suspended solids and do not have a tendency to form a surface film under test; or

(ii) Pensky-Martens Closed Tester (see American National Standard Method of Test for Flash Point by Pensky-Martens Closed Tester, Z11.7-1979 (ASTM D 93-79)) for liquids with a viscosity equal to or greater than 45 SUS at 100 deg. F (37.8 deg. C), or that contain suspended solids, or that have a tendency to form a surface film under test; or

(iii) Setaflash Closed Tester (see American National Standard Method of Test for Flash Point by Setaflash Closed Tester (ASTM D 3278-78)).

Organic peroxides, which undergo auto accelerating thermal decomposition, are excluded from any of the flashpoint determination methods specified above.

"Foreseeable emergency" means any potential occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment which could result in an uncontrolled release of a hazardous chemical into the workplace. 25

"Hazardous chemical" means any chemical which is a physical hazard or a health hazard.

"Hazard warning" means any words, pictures, symbols, or combination thereof appearing on a label or other appropriate form of warning which convey the specific physical and health hazard(s), including target organ effects, of the chemical(s) in the container(s). (See the definitions for "physical hazard" and "health hazard" to determine the hazards which must be covered.)

"Health hazard" means a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees. The term "health hazard" includes chemicals which are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents which act on the hematopoietic system and agents which damage the lungs, skin, eyes, or mucous membranes. Appendix A provides further definitions and explanations of the scope of health hazards covered by this section, and Appendix B describes the criteria to be used to determine whether or not a chemical is to be considered hazardous for purposes of this standard.

"Identity" means any chemical or common name which is indicated on the material safety data sheet (MSDS) for the chemical. The identity used shall permit cross-references to be made among the required list of hazardous chemicals, the label and the MSDS.

"Immediate use" means that the hazardous chemical will be under the control of and used only by the person who transfers it from a labeled container and only within the work shift in which it is transferred.

"Importer" means the first business with employees within the Customs Territory of the United States which receives hazardous chemicals produced in other countries for the purpose of supplying them to distributors or employers within the United States.

"Label" means any written, printed, or graphic material displayed on or affixed to containers of hazardous chemicals.

"Material safety data sheet (MSDS)" means written or printed material concerning a hazardous chemical which is prepared in accordance with paragraph (g) of this section.

"Mixture" means any combination of two or more chemicals if the combination is not, in whole or in part, the result of a chemical reaction.

"Organic peroxide" means an organic compound that contains the bivalent -O-O-structure and which may be considered to be a structural derivative of hydrogen peroxide where one or both of the hydrogen atoms has been replaced by an organic radical.

"Oxidizer" means a chemical other than a blasting agent or explosive as defined in 1910.109(a), that initiates or promotes combustion in other materials, thereby causing fire either of itself or through the release of oxygen or other gases.

"Physical hazard" means a chemical for which there is scientifically valid evidence that it is a combustible liquid, a compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable (reactive) or water-reactive.



"Produce" means to manufacture, process, formulate, blend, extract, generate, emit, or repackage.

"Pyrophoric" means a chemical that will ignite spontaneously in air at a temperature of 130 deg. F (54.4 deg. C) or below.

"Responsible party" means someone who can provide additional information on the hazardous chemical and appropriate emergency procedures, if necessary.

"Specific chemical identity" means the chemical name, Chemical Abstracts Service (CAS) Registry Number, or any other information that reveals the precise chemical designation of the substance.

"Trade secret" means any confidential formula, pattern, process, device, information or compilation of information that is used in an employer's business, and that gives the employer an opportunity to obtain an advantage over competitors who do not know or use it. Appendix D sets out the criteria to be used in evaluating trade secrets.

"Unstable (reactive)" means a chemical which in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shocks, pressure or temperature. 26

"Use" means to package, handle, react, emit, extract, generate as a byproduct, or transfer.

"Water-reactive" means a chemical that reacts with water to release a gas that is either flammable or presents a health hazard.

"Work area" means a room or defined space in a workplace where hazardous chemicals are produced or used, and where employees are present.

"Workplace" means an establishment, job site, or project, at one geographical location containing one or more work areas.

**..1910.1200(d)**

**1910.1200(d)**

"Hazard determination."

**1910.1200(d)(1)**

Chemical manufacturers and importers shall evaluate chemicals produced in their workplaces or imported by them to determine if they are hazardous. Employers are not required to evaluate chemicals unless they choose not to rely on the evaluation performed by the chemical manufacturer or importer for the chemical to satisfy this requirement.

**1910.1200(d)(2)**

Chemical manufacturers, importers or employers evaluating chemicals shall identify and consider the available scientific evidence concerning such hazards. For health hazards, evidence which is statistically significant and which is based on at least one positive study conducted in accordance with established scientific principles is considered to be sufficient to establish a hazardous effect if the results of the study meet the definitions of health hazards in this section. Appendix A shall be consulted for the scope of health hazards covered, and Appendix B shall be consulted for the criteria to be followed with respect to the completeness of the evaluation, and the data to be reported.

**1910.1200(d)(3)**

The chemical manufacturer, importer or employer evaluating chemicals shall treat the following sources as establishing that the chemicals listed in them are hazardous:

**1910.1200(d)(3)(i)**

29 CFR part 1910, subpart Z, Toxic and Hazardous Substances, Occupational Safety and Health Administration (OSHA); or,

**..1910.1200(d)(3)(ii)**

**1910.1200(d)(3)(ii)**

"Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment," American Conference of Governmental Industrial Hygienists (ACGIH) (latest edition). The chemical manufacturer, importer, or employer is still responsible for evaluating the hazards associated with the chemicals in these source lists in accordance with the requirements of this standard.

**1910.1200(d)(4)**

Chemical manufacturers, importers and employers evaluating chemicals shall treat the following sources as establishing that a chemical is a carcinogen or potential carcinogen for hazard communication purposes:

**1910.1200(d)(4)(i)**

National Toxicology Program (NTP), "Annual Report on Carcinogens" (latest edition);

**1910.1200(d)(4)(ii)**

International Agency for Research on Cancer (IARC) "Monographs" (latest editions); or

**1910.1200(d)(4)(iii)**

29 CFR part 1910, subpart Z, Toxic and Hazardous Substances, Occupational Safety and Health Administration.

Note: The "Registry of Toxic Effects of Chemical Substances" published by the National Institute for Occupational Safety and Health indicates whether a chemical has been found by NTP or IARC to be a potential carcinogen.

**1910.1200(d)(5)**

The chemical manufacturer, importer or employer shall determine the hazards of mixtures of chemicals as follows: 27

**1910.1200(d)(5)(i)**

If a mixture has been tested as a whole to determine its hazards, the results of such testing shall be used to determine whether the mixture is hazardous;

**..1910.1200(d)(5)(ii)**

**1910.1200(d)(5)(ii)**

If a mixture has not been tested as a whole to determine whether the mixture is a health hazard, the mixture shall be assumed to present the same health hazards as do the components which comprise one percent (by weight or volume) or greater of the mixture, except that the mixture shall be assumed to present a carcinogenic hazard if it contains a component in concentrations of 0.1 percent or greater which is considered to be a carcinogen under paragraph (d)(4) of this section;

**1910.1200(d)(5)(iii)**

If a mixture has not been tested as a whole to determine whether the mixture is a physical hazard, the chemical manufacturer, importer, or employer may use whatever scientifically valid data is available to evaluate the physical hazard potential of the mixture; and,

**1910.1200(d)(5)(iv)**

If the chemical manufacturer, importer, or employer has evidence to indicate that a component present in the mixture in concentrations of less than one percent (or in the case of carcinogens,

less than 0.1 percent) could be released in concentrations which would exceed an established OSHA permissible exposure limit or ACGIH Threshold Limit Value, or could present a health risk to employees in those concentrations, the mixture shall be assumed to present the same hazard.

**1910.1200(d)(6)**

Chemical manufacturers, importers, or employers evaluating chemicals shall describe in writing the procedures they use to determine the hazards of the chemical they evaluate. The written procedures are to be made available, upon request, to employees, their designated representatives, the Assistant Secretary and the Director. The written description may be incorporated into the written hazard communication program required under paragraph (e) of this section.

**..1910.1200(e)**

**1910.1200(e)**

"Written hazard communication program."

**1910.1200(e)(1)**

Employers shall develop, implement, and maintain at each workplace, a written hazard communication program which at least describes how the criteria specified in paragraphs (f), (g), and (h) of this section for labels and other forms of warning, material safety data sheets, and employee information and training will be met, and which also includes the following:

**1910.1200(e)(1)(i)**

A list of the hazardous chemicals known to be present using an identity that is referenced on the appropriate material safety data sheet (the list may be compiled for the workplace as a whole or for individual work areas); and,

**1910.1200(e)(1)(ii)**

The methods the employer will use to inform employees of the hazards of non-routine tasks (for example, the cleaning of reactor vessels), and the hazards associated with chemicals contained in unlabeled pipes in their work areas.

**1910.1200(e)(2)**

"Multi-employer workplaces." Employers who produce, use, or store hazardous chemicals at a workplace in such a way that the employees of other employer(s) may be exposed (for example, employees of a construction contractor working on-site) shall additionally ensure that the hazard communication programs developed and implemented under this paragraph (e) include the following:

**1910.1200(e)(2)(i)**

The methods the employer will use to provide the other employer(s) on-site access to material safety data sheets for each hazardous chemical the other employer(s)' employees may be exposed to while working;

**..1910.1200(e)(2)(ii) 28**

**1910.1200(e)(2)(ii)**

The methods the employer will use to inform the other employer(s) of any precautionary measures that need to be taken to protect employees during the workplace's normal operating conditions and in foreseeable emergencies; and,

**1910.1200(e)(2)(iii)**

The methods the employer will use to inform the other employer(s) of the labeling system used in the workplace.

**1910.1200(e)(3)**

The employer may rely on an existing hazard communication program to comply with these requirements, provided that it meets the criteria established in this paragraph (e).

**1910.1200(e)(4)**

The employer shall make the written hazard communication program available, upon request, to employees, their designated representatives, the Assistant Secretary and the Director, in accordance with the requirements of 29 CFR 1910.1020 (e).

**1910.1200(e)(5)**

Where employees must travel between workplaces during a workshift, i.e., their work is carried out at more than one geographical location, the written hazard communication program may be kept at the primary workplace facility.

**1910.1200(f)**

"Labels and other forms of warning."

**1910.1200(f)(1)**

The chemical manufacturer, importer, or distributor shall ensure that each container of hazardous chemicals leaving the workplace is labeled, tagged or marked with the following information:

**..1910.1200(f)(1)(i)**

**1910.1200(f)(1)(i)**

Identity of the hazardous chemical(s);

**1910.1200(f)(1)(ii)**

Appropriate hazard warnings; and

**1910.1200(f)(1)(iii)**

Name and address of the chemical manufacturer, importer, or other responsible party.

**1910.1200(f)(2)**

**1910.1200(f)(2)(i)**

For solid metal (such as a steel beam or a metal casting), solid wood, or plastic items that are not exempted as articles due to their downstream use, or shipments of whole grain, the required label may be transmitted to the customer at the time of the initial shipment, and need not be included with subsequent shipments to the same employer unless the information on the label changes;

**1910.1200(f)(2)(ii)**

The label may be transmitted with the initial shipment itself, or with the material safety data sheet that is to be provided prior to or at the time of the first shipment; and,

**1910.1200(f)(2)(iii)**

This exception to requiring labels on every container of hazardous chemicals is only for the solid material itself, and does not apply to hazardous chemicals used in conjunction with, or known to be present with, the material and to which employees handling the items in transit may be exposed (for example, cutting fluids or pesticides in grains).

**..1910.1200(f)(3)**

**1910.1200(f)(3)**

Chemical manufacturers, importers, or distributors shall ensure that each container of hazardous chemicals leaving the workplace is labeled, tagged, or marked in accordance with this section in a manner which does not conflict with the requirements of the Hazardous Materials Transportation Act (49 U.S.C. 1801 et seq.) and regulations issued under that Act by the Department of Transportation.

**1910.1200(f)(4)** If the hazardous chemical is regulated by OSHA in a substance-specific health standard, the chemical manufacturer, importer, distributor or employer shall ensure that the labels or other forms of warning used are in accordance with the requirements of that standard.

**1910.1200(f)(5)**

Except as provided in paragraphs (f)(6) and (f)(7) of this section, the employer shall ensure that each container of hazardous chemicals in the workplace is labeled, tagged or marked with the following information:

**1910.1200(f)(5)(i)**

Identity of the hazardous chemical(s) contained therein; and,

**1910.1200(f)(5)(ii)**

Appropriate hazard warnings, or alternatively, words, pictures, symbols, or combination thereof, which provide at least general information regarding the hazards of the chemicals, and which, in conjunction with the other information immediately available to employees under the hazard communication program, will provide employees with the specific information regarding the physical and health hazards of the hazardous chemical.

**1910.1200(f)(6)**

**1910.1200(f)(6)**

The employer may use signs, placards, process sheets, batch tickets, operating procedures, or other such written materials in lieu of affixing labels to individual stationary process containers, as long as the alternative method identifies the containers to which it is applicable and conveys the information required by paragraph (f)(5) of this section to be on a label. The written materials shall be readily accessible to the employees in their work area throughout each work shift.

**1910.1200(f)(7)**

The employer is not required to label portable containers into which hazardous chemicals are transferred from labeled containers, and which are intended only for the immediate use of the employee who performs the transfer. For purposes of this section, drugs which are dispensed by a pharmacy to a health care provider for direct administration to a patient are exempted from labeling.

**1910.1200(f)(8)**

The employer shall not remove or deface existing labels on incoming containers of hazardous chemicals, unless the container is immediately marked with the required information.

**1910.1200(f)(9)**

The employer shall ensure that labels or other forms of warning are legible, in English, and prominently displayed on the container, or readily available in the work area throughout each work shift. Employers having employees who speak other languages may add the information in their language to the material presented, as long as the information is presented in English as well.

**1910.1200(f)(10)**

The chemical manufacturer, importer, distributor or employer need not affix new labels to comply with this section if existing labels already convey the required information.

**1910.1200(f)(11)**

**1910.1200(f)(11)**

Chemical manufacturers, importers, distributors, or employers who become newly aware of any significant information regarding the hazards of a chemical shall revise the labels for the chemical within three months of becoming aware of the new information. Labels on containers

of hazardous chemicals shipped after that time shall contain the new information. If the chemical is not currently produced or imported, the chemical manufacturer, importers, distributor, or employer shall add the information to the label before the chemical is shipped or introduced into the workplace again.

**1910.1200(g)**

"Material safety data sheets."

**1910.1200(g)(1)**

Chemical manufacturers and importers shall obtain or develop a material safety data sheet for each hazardous chemical they produce or import. Employers shall have a material safety data sheet in the workplace for each hazardous chemical which they use.

**1910.1200(g)(2)** 30 Each material safety data sheet shall be in English (although the employer may maintain copies in other languages as well), and shall contain at least the following information:

**1910.1200(g)(2)(i)**

The identity used on the label, and, except as provided for in paragraph (i) of this section on trade secrets:

**1910.1200(g)(2)(i)(A)**

If the hazardous chemical is a single substance, its chemical and common name(s);

**1910.1200(g)(2)(i)(B)**

If the hazardous chemical is a mixture which has been tested as a whole to determine its hazards, the chemical and common name(s) of the ingredients which contribute to these known hazards, and the common name(s) of the mixture itself; or,

**1910.1200(g)(2)(i)(C)**

If the hazardous chemical is a mixture which has not been tested as a whole:

**..1910.1200(g)(2)(i)(C)(1)**

**1910.1200(g)(2)(i)(C)(1)**

The chemical and common name(s) of all ingredients which have been determined to be health hazards, and which comprise 1% or greater of the composition, except that chemicals identified as carcinogens under paragraph (d) of this section shall be listed if the concentrations are 0.1% or greater; and,

**1910.1200(g)(2)(i)(C)(2)**

The chemical and common name(s) of all ingredients which have been determined to be health hazards, and which comprise less than 1% (0.1% for carcinogens) of the mixture, if there is evidence that the ingredient(s) could be released from the mixture in concentrations which would exceed an established OSHA permissible exposure limit or ACGIH Threshold Limit Value, or could present a health risk to employees; and,

**1910.1200(g)(2)(i)(C)(3)**

The chemical and common name(s) of all ingredients which have been determined to present a physical hazard when present in the mixture;

**1910.1200(g)(2)(ii)**

Physical and chemical characteristics of the hazardous chemical (such as vapor pressure, flash point);

**1910.1200(g)(2)(iii)**

The physical hazards of the hazardous chemical, including the potential for fire, explosion, and reactivity;

**1910.1200(g)(2)(iv)**

The health hazards of the hazardous chemical, including signs and symptoms of exposure, and any medical conditions which are generally recognized as being aggravated by exposure to the chemical;

**1910.1200(g)(2)(v)**

The primary route(s) of entry;

**..1910.1200(g)(2)(vi)**

**1910.1200(g)(2)(vi)**

The OSHA permissible exposure limit, ACGIH Threshold Limit Value, and any other exposure limit used or recommended by the chemical manufacturer, importer, or employer preparing the material safety data sheet, where available;

**1910.1200(g)(2)(vii)**

Whether the hazardous chemical is listed in the National Toxicology Program (NTP) Annual Report on Carcinogens (latest edition) or has been found to be a potential carcinogen in the International Agency for Research on Cancer (IARC) Monographs (latest editions), or by OSHA;

**1910.1200(g)(2)(viii)**

Any generally applicable precautions for safe handling and use which are known to the chemical manufacturer, importer or employer preparing the material safety data sheet, including appropriate hygienic practices, protective measures during repair and maintenance of contaminated equipment, and procedures for clean-up of spills and leaks; 31

**1910.1200(g)(2)(ix)**

Any generally applicable control measures which are known to the chemical manufacturer, importer or employer preparing the material safety data sheet, such as appropriate engineering controls, work practices, or personal protective equipment;

**1910.1200(g)(2)(x)**

Emergency and first aid procedures;

**1910.1200(g)(2)(xi)**

The date of preparation of the material safety data sheet or the last change to it; and,

**..1910.1200(g)(2)(xii)**

**1910.1200(g)(2)(xii)**

The name, address and telephone number of the chemical manufacturer, importer, employer or other responsible party preparing or distributing the material safety data sheet, who can provide additional information on the hazardous chemical and appropriate emergency procedures, if necessary.

**1910.1200(g)(3)**

If no relevant information is found for any given category on the material safety data sheet, the chemical manufacturer, importer or employer preparing the material safety data sheet shall mark it to indicate that no applicable information was found.

**1910.1200(g)(4)**

Where complex mixtures have similar hazards and contents (i.e. the chemical ingredients are essentially the same, but the specific composition varies from mixture to mixture), the chemical manufacturer, importer or employer may prepare one material safety data sheet to apply to all of these similar mixtures.

**1910.1200(g)(5)**

The chemical manufacturer, importer or employer preparing the material safety data sheet shall ensure that the information recorded accurately reflects the scientific evidence used in making the hazard determination. If the chemical manufacturer, importer or employer preparing the material safety data sheet becomes newly aware of any significant information regarding the hazards of a chemical, or ways to protect against the hazards, this new information shall be added to the material safety data sheet within three months. If the chemical is not currently being produced or imported the chemical manufacturer or importer shall add the information to the material safety data sheet before the chemical is introduced into the workplace again.

**..1910.1200(g)(6)**

**1910.1200(g)(6)**

**1910.1200(g)(6)(i)**

Chemical manufacturers or importers shall ensure that distributors and employers are provided an appropriate material safety data sheet with their initial shipment, and with the first shipment after a material safety data sheet is updated;

**1910.1200(g)(6)(ii)**

The chemical manufacturer or importer shall either provide material safety data sheets with the shipped containers or send them to the distributor or employer prior to or at the time of the shipment;

**1910.1200(g)(6)(iii)**

If the material safety data sheet is not provided with a shipment that has been labeled as a hazardous chemical, the distributor or employer shall obtain one from the chemical manufacturer or importer as soon as possible; and,

**1910.1200(g)(6)(iv)**

The chemical manufacturer or importer shall also provide distributors or employers with a material safety data sheet upon request.

**1910.1200(g)(7)**

**1910.1200(g)(7)(i)**

Distributors shall ensure that material safety data sheets, and updated information, are provided to other distributors and employers with their initial shipment and with the first shipment after a material safety data sheet is updated; 32

**1910.1200(g)(7)(ii)**

The distributor shall either provide material safety data sheets with the shipped containers, or send them to the other distributor or employer prior to or at the time of the shipment;

**..1910.1200(g)(7)(iii)**

**1910.1200(g)(7)(iii)**

Retail distributors selling hazardous chemicals to employers having a commercial account shall provide a material safety data sheet to such employers upon request, and shall post a sign or otherwise inform them that a material safety data sheet is available;

**1910.1200(g)(7)(iv)**

Wholesale distributors selling hazardous chemicals to employers over-the-counter may also provide material safety data sheets upon the request of the employer at the time of the over-the-counter purchase, and shall post a sign or otherwise inform such employers that a material safety data sheet is available;

**1910.1200(g)(7)(v)**



If an employer without a commercial account purchases a hazardous chemical from a retail distributor not required to have material safety data sheets on file (i.e., the retail distributor does not have commercial accounts and does not use the materials), the retail distributor shall provide the employer, upon request, with the name, address, and telephone number of the chemical manufacturer, importer, or distributor from which a material safety data sheet can be obtained;

**1910.1200(g)(7)(vi)**

Wholesale distributors shall also provide material safety data sheets to employers or other distributors upon request; and,

**1910.1200(g)(7)(vii)**

Chemical manufacturers, importers, and distributors need not provide material safety data sheets to retail distributors that have informed them that the retail distributor does not sell the product to commercial accounts or open the sealed container to use it in their own workplaces.

**..1910.1200(g)(8)**

**1910.1200(g)(8)**

The employer shall maintain in the workplace copies of the required material safety data sheets for each hazardous chemical, and shall ensure that they are readily accessible during each work shift to employees when they are in their work area(s). (Electronic access, microfiche, and other alternatives to maintaining paper copies of the material safety data sheets are permitted as long as no barriers to immediate employee access in each workplace are created by such options.)

**1910.1200(g)(9)**

Where employees must travel between workplaces during a workshift, i.e., their work is carried out at more than one geographical location, the material safety data sheets may be kept at the primary workplace facility. In this situation, the employer shall ensure that employees can immediately obtain the required information in an emergency.

**1910.1200(g)(10)**

Material safety data sheets may be kept in any form, including operating procedures, and may be designed to cover groups of hazardous chemicals in a work area where it may be more appropriate to address the hazards of a process rather than individual hazardous chemicals. However, the employer shall ensure that in all cases the required information is provided for each hazardous chemical, and is readily accessible during each work shift to employees when they are in in their work area(s).

**1910.1200(g)(11)**

Material safety data sheets shall also be made readily available, upon request, to designated representatives and to the Assistant Secretary, in accordance with the requirements of 29 CFR 1910.1020(e). The Director shall also be given access to material safety data sheets in the same manner.

**..1910.1200(h)**

**1910.1200(h) 33**

"Employee information and training."

**1910.1200(h)(1)**

Employers shall provide employees with effective information and training on hazardous chemicals in their work area at the time of their initial assignment, and whenever a new physical or health hazard the employees have not previously been trained about is introduced into their work area. Information and training may be designed to cover categories of hazards (e.g.,

flammability, carcinogenicity) or specific chemicals. Chemical-specific information must always be available through labels and material safety data sheets.

**1910.1200(h)(2)**

"Information." Employees shall be informed of:

**1910.1200(h)(2)(i)**

The requirements of this section;

**1910.1200(h)(2)(ii)**

Any operations in their work area where hazardous chemicals are present; and,

**1910.1200(h)(2)(iii)**

The location and availability of the written hazard communication program, including the required list(s) of hazardous chemicals, and material safety data sheets required by this section.

**1910.1200(h)(3)**

"Training." Employee training shall include at least:

**1910.1200(h)(3)(i)**

Methods and observations that may be used to detect the presence or release of a hazardous chemical in the work area (such as monitoring conducted by the employer, continuous monitoring devices, visual appearance or odor of hazardous chemicals when being released, etc.);

**1910.1200(h)(3)(ii)**

The physical and health hazards of the chemicals in the work area;

**1910.1200(h)(3)(iii)**

**1910.1200(h)(3)(iii)**

The measures employees can take to protect themselves from these hazards, including specific procedures the employer has implemented to protect employees from exposure to hazardous chemicals, such as appropriate work practices, emergency procedures, and personal protective equipment to be used; and,

**1910.1200(h)(3)(iv)**

The details of the hazard communication program developed by the employer, including an explanation of the labeling system and the material safety data sheet, and how employees can obtain and use the appropriate hazard information.

**1910.1200(i)**

"Trade secrets."

**1910.1200(i)(1)**

The chemical manufacturer, importer, or employer may withhold the specific chemical identity, including the chemical name and other specific identification of a hazardous chemical, from the material safety data sheet, provided that:

**1910.1200(i)(1)(i)**

The claim that the information withheld is a trade secret can be supported;

**1910.1200(i)(1)(ii)**

Information contained in the material safety data sheet concerning the properties and effects of the hazardous chemical is disclosed;

**1910.1200(i)(1)(iii)**

The material safety data sheet indicates that the specific chemical identity is being withheld as a trade secret; and,

**1910.1200(i)(1)(iv)**

The specific chemical identity is made available to health professionals, employees, and designated representatives in accordance with the applicable provisions of this paragraph. 34

**..1910.1200(i)(2)**

**1910.1200(i)(2)**

Where a treating physician or nurse determines that a medical emergency exists and the specific chemical identity of a hazardous chemical is necessary for emergency or first-aid treatment, the chemical manufacturer, importer, or employer shall immediately disclose the specific chemical identity of a trade secret chemical to that treating physician or nurse, regardless of the existence of a written statement of need or a confidentiality agreement. The chemical manufacturer, importer, or employer may require a written statement of need and confidentiality agreement, in accordance with the provisions of paragraphs (i)(3) and (4) of this section, as soon as circumstances permit.

**1910.1200(i)(3)**

In non-emergency situations, a chemical manufacturer, importer, or employer shall, upon request, disclose a specific chemical identity, otherwise permitted to be withheld under paragraph (i)(1) of this section, to a health professional (i.e. physician, industrial hygienist, toxicologist, epidemiologist, or occupational health nurse) providing medical or other occupational health services to exposed employee(s), and to employees or designated representatives, if:

**1910.1200(i)(3)(i)**

The request is in writing;

**1910.1200(i)(3)(ii)**

The request describes with reasonable detail one or more of the following occupational health needs for the information:

**1910.1200(i)(3)(ii)(A)**

To assess the hazards of the chemicals to which employees will be exposed;

**1910.1200(i)(3)(ii)(B)**

To conduct or assess sampling of the workplace atmosphere to determine employee exposure levels;

**1910.1200(i)(3)(ii)(C)**

To conduct pre-assignment or periodic medical surveillance of exposed employees;

**1910.1200(i)(3)(ii)(D)**

To provide medical treatment to exposed employees;

**..1910.1200(i)(3)(ii)(E)**

**1910.1200(i)(3)(ii)(E)**

To select or assess appropriate personal protective equipment for exposed employees;

**1910.1200(i)(3)(ii)(F)**

To design or assess engineering controls or other protective measures for exposed employees; and,

**1910.1200(i)(3)(ii)(G)**

To conduct studies to determine the health effects of exposure.

**1910.1200(i)(3)(iii)**

The request explains in detail why the disclosure of the specific chemical identity is essential and that, in lieu thereof, the disclosure of the following information to the health professional,

employee, or designated representative, would not satisfy the purposes described in paragraph (i)(3)(ii) of this section:

**1910.1200(i)(3)(iii)(A)**

The properties and effects of the chemical;

**1910.1200(i)(3)(iii)(B)**

Measures for controlling workers' exposure to the chemical;

**1910.1200(i)(3)(iii)(C)**

Methods of monitoring and analyzing worker exposure to the chemical; and,

**1910.1200(i)(3)(iii)(D)**

Methods of diagnosing and treating harmful exposures to the chemical;

**1910.1200(i)(3)(iv)**

The request includes a description of the procedures to be used to maintain the confidentiality of the disclosed information; and,

**..1910.1200(i)(3)(v) 351910.1200(i)(3)(v)**

The health professional, and the employer or contractor of the services of the health professional (i.e. downstream employer, labor organization, or individual employee), employee, or designated representative, agree in a written confidentiality agreement that the health professional, employee, or designated representative, will not use the trade secret information for any purpose other than the health need(s) asserted and agree not to release the information under any circumstances other than to OSHA, as provided in paragraph (i)(6) of this section, except as authorized by the terms of the agreement or by the chemical manufacturer, importer, or employer.

**1910.1200(i)(4)**

The confidentiality agreement authorized by paragraph (i)(3)(iv) of this section:

**1910.1200(i)(4)(i)**

May restrict the use of the information to the health purposes indicated in the written statement of need;

**1910.1200(i)(4)(ii)**

May provide for appropriate legal remedies in the event of a breach of the agreement, including stipulation of a reasonable pre-estimate of likely damages; and,

**1910.1200(i)(4)(iii)**

May not include requirements for the posting of a penalty bond.

**1910.1200(i)(5)**

Nothing in this standard is meant to preclude the parties from pursuing non-contractual remedies to the extent permitted by law.

**1910.1200(i)(6)**

If the health professional, employee, or designated representative receiving the trade secret information decides that there is a need to disclose it to OSHA, the chemical manufacturer, importer, or employer who provided the information shall be informed by the health professional, employee, or designated representative prior to, or at the same time as, such disclosure.

**..1910.1200(i)(7)**

**1910.1200(i)(7)**

If the chemical manufacturer, importer, or employer denies a written request for disclosure of a specific chemical identity, the denial must:

**1910.1200(i)(7)(i)**

Be provided to the health professional, employee, or designated representative, within thirty days of the request;

**1910.1200(i)(7)(ii)**

Be in writing;

**1910.1200(i)(7)(iii)**

Include evidence to support the claim that the specific chemical identity is a trade secret;

**1910.1200(i)(7)(iv)**

State the specific reasons why the request is being denied; and,

**1910.1200(i)(7)(v)**

Explain in detail how alternative information may satisfy the specific medical or occupational health need without revealing the specific chemical identity.

**1910.1200(i)(8)**

The health professional, employee, or designated representative whose request for information is denied under paragraph (i)(3) of this section may refer the request and the written denial of the request to OSHA for consideration.

**1910.1200(i)(9)**

When a health professional, employee, or designated representative refers the denial to OSHA under paragraph (i)(8) of this section, OSHA shall consider the evidence to determine if:

**..1910.1200(i)(9)(i)**

**1910.1200(i)(9)(i)**

The chemical manufacturer, importer, or employer has supported the claim that the specific chemical identity is a trade secret;

**1910.1200(i)(9)(ii)**

The health professional, employee, or designated representative has supported the claim that there is a medical or occupational health need for the information; and,

**1910.1200(i)(9)(iii)**

The health professional, employee or designated representative has demonstrated adequate means to protect the confidentiality.

**1910.1200(i)(10)**

**1910.1200(i)(10)(i)**

If OSHA determines that the specific chemical identity requested under paragraph (i)(3) of this section is not a "bona fide" trade secret, or that it is a trade secret, but the requesting health professional, employee, or designated representative has a legitimate medical or occupational health need for the information, has executed a written confidentiality agreement, and has shown adequate means to protect the confidentiality of the information, the chemical manufacturer, importer, or employer will be subject to citation by OSHA.

**..1910.1200(i)(10)(ii)**

**1910.1200(i)(10)(ii)**

If a chemical manufacturer, importer, or employer demonstrates to OSHA that the execution of a confidentiality agreement would not provide sufficient protection against the potential harm from the unauthorized disclosure of a trade secret specific chemical identity, the Assistant Secretary may issue such orders or impose such additional limitations or conditions upon the disclosure of the requested chemical information as may be appropriate to assure that the occupational health

services are provided without an undue risk of harm to the chemical manufacturer, importer, or employer.

**1910.1200(i)(11)**

If a citation for a failure to release specific chemical identity information is contested by the chemical manufacturer, importer, or employer, the matter will be adjudicated before the Occupational Safety and Health Review Commission in accordance with the Act's enforcement scheme and the applicable Commission rules of procedure. In accordance with the Commission rules, when a chemical manufacturer, importer, or employer continues to withhold the information during the contest, the Administrative Law Judge may review the citation and supporting documentation "in camera" or issue appropriate orders to protect the confidentiality of such matters.

**1910.1200(i)(12)**

Notwithstanding the existence of a trade secret claim, a chemical manufacturer, importer, or employer shall, upon request, disclose to the Assistant Secretary any information which this section requires the chemical manufacturer, importer, or employer to make available. Where there is a trade secret claim, such claim shall be made no later than at the time the information is provided to the Assistant Secretary so that suitable determinations of trade secret status can be made and the necessary protections can be implemented.

**1910.1200(i)(13)**

Nothing in this paragraph shall be construed as requiring the disclosure under any circumstances of process or percentage of mixture information which is a trade secret.

**..1910.1200(j)**

**1910.1200(j)**

"Effective dates." Chemical manufacturers, importers, distributors, and employers shall be in compliance with all provisions of this section by March 11, 1994.

Note: The effective date of the clarification that the exemption of wood and wood products from the Hazard Communication standard in paragraph (b)(6)(iv) only applies to wood and wood products including lumber which will not be processed, where the manufacturer or importer can establish that the only hazard they pose to employees is the potential for flammability or combustibility, and that the exemption does not apply to wood or wood products which have been treated with a hazardous chemical covered by this standard, and wood which may be subsequently sawed or cut generating dust has been stayed from March 11, 1994 to August 11, 1994. 37

[59 FR 17479, April 13, 1994; 59 FR 65947, Dec. 22, 1994; 61 FR 5507, Feb. 13, 1996] 38

# **Appendix C**

## **HMIS Labeling System**

Example of the HMIS Label:

Chemical Name	
CAS#	
<b>HEALTH</b>	<input type="checkbox"/>
<b>FLAMMABILITY</b>	<input type="checkbox"/>
<b>REACTIVITY</b>	<input type="checkbox"/>
<b>SPECIFIC</b>	<input type="checkbox"/>

<b>HEALTH</b>	<b>FLAMMABILITY</b>	<b>REACTIVITY</b>
<b>4:</b> Deadly: even the slightest exposure to this substance would be life threatening. Only specialized protective clothing, for these materials, should be worn.	<b>4:</b> Flash Point Below 73°F and Boiling Point Below 100°F: this substance is very flammable, volatile or explosive depending on its state. Extreme caution should be used in handling or storing of these 3:materials.	<b>4:</b> May Detonate: substances that are readily capable of detonation or explosion at normal temperatures and pressures. Evacuate area if exposed to heat or fire.
<b>3:</b> Extreme Danger: serious injury would result from exposure to this substance. Do not expose any body surface to these materials. Full protective measures should be taken.	<b>3:</b> Flash Point Below 100°F: flammable, volatile or explosive under almost all normal temperature conditions. Exercise great caution in storage or handling of these materials.	<b>3:</b> Explosive: substances that are readily capable of detonation or explosion by a strong initiating source, such as heat, shock or water. Monitor from behind explosion-resistant barriers.
<b>2:</b> Dangerous: exposure to this substance would be hazardous to health. Protective measures are indicated.	<b>2:</b> Flash Point Below 200°F: moderately heated conditions may ignite this substance. Caution procedures should be employed in handling.	<b>2:</b> Unstable: violent chemical changes are possible at normal or elevated temperatures and pressures. Potentially violent or explosive reaction may occur when mixed with water. Monitor from a safe distance.
<b>1:</b> Slight Hazard: irritation or minor injury would result from exposure to this substance. Protective measures are indicated.	<b>1:</b> Flash Point Above 200°F: this substance must be preheated to ignite. Most combustible solids would be in this category.	<b>1:</b> Normally stable: substances that may become unstable at elevated temperatures and pressures or when mixed with water. Approach with caution.
<b>0:</b> No Hazard: exposure to this substance offers no significant risk to health.	<b>0:</b> Will Not Burn: substances that will not burn.	<b>0:</b> Stable: substances which will remain stable when exposed to heat, pressure or water.