

IBC-SOP-001

Title: NIH Guidelines Biosafety Requirements

Effective Date: 21 October 2015

Revision History:

Purpose

The purpose of this document is to provide the information needed to meet the various biosafety requirements to ensure compliance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. This document provides general MSU procedures. Each researcher can use this document to customize to individual research programs.

Procedure

Biosafety requirements for research involving recombinant/synthetic nucleic acid molecules include the following:

1. Principal Investigator (PI) Responsibilities

On behalf of the institution, the PI is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant or synthetic nucleic acid molecule research. The PI is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

General Responsibilities: As part of this general responsibility, the PI shall:

1. Initiate or modify no recombinant or synthetic nucleic acid molecule research which requires Institutional Biosafety Committee approval until that research or the proposed modification has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;
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;
3. 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));
4. Be adequately trained in good microbiological techniques;
5. Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination; and
6. Comply with shipping requirements for recombinant or synthetic nucleic acid molecules (see [Appendix H](#), *Shipment*, for shipping requirements and the *Laboratory Safety Monograph* for technical recommendations).

Information to Be Submitted by the PI to NIH OBA

The Principal Investigator shall:

1. Submit information to NIH/OBA for certification of new host-vector systems;
2. Petition NIH/OBA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the *NIH Guidelines*;

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*;
4. Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and
5. Petition NIH/OBA for determination of containment for experiments not covered by the *NIH Guidelines*.

Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*
2. Select appropriate microbiological practices and laboratory techniques to be used for the research;
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
4. Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

1. Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
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
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

1. Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
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));
3. Correct work errors and conditions that may result in the release of recombinant or synthetic nucleic acid molecule materials; and

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).

2. PI Training Mechanisms for the *NIH Guidelines*

PIs can become familiar with the *NIH Guidelines* requirements through any number of ways:

- On-line class available through myCourses
- Web page containing information specific to the *NIH Guidelines*
- NIH page
- Forms page
- PIs receive an annual email blast
- PIs receive information at faculty meeting by RC personnel
- IBC-SOP-001 (this SOP)
- Contact BSO

3. Incident Reporting

The PI is required to report any

1. significant problems,
2. violations of the *NIH Guidelines*,
3. or any significant research-related accidents and illnesses

to the Biological Safety Officer, 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 address listed in Section IV-B-7-2-(2) of the *Guidelines*.

Certain incidents require reporting within **24 hours**. These include:

Incidents occurring under BSL-2 conditions that result in an OVERT exposure to organisms containing recombinant or synthetic nucleic acid molecules

IBC-PP-010 and IBC-PP-016 contain the specific policies for incident reporting.

4.Recombinant/Synthetic Nucleic Acid Waste Disposal

Recombinant or synthetic nucleic acid molecules are considered biohazardous waste. This section describes how this waste is disposed of.

- a. All waste containing recombinant or synthetic nucleic acid molecules must either be decontaminated on site or removed by MSU's contracted medical waste vendor.
- b. Organisms containing recombinant or synthetic nucleic acid molecules must be contained by physical or biological methods. Proper disposal of waste is part of physical containment.
- c. Each biosafety level has specific practices:

- i. At BSL-1
 1. all contaminated liquid or solid wastes are decontaminated before disposal and
 2. contaminated materials that are to be decontaminated at a site away from the lab are placed in a durable, leak-proof container which is closed before removal from the lab.
- ii. At BSL-2, includes all BSL-1 practices plus
 1. All wastes from labs and animal rooms are appropriately contaminated
 2. An autoclave is available for decontamination.

Sharps: DO NOT place any kind of sharps in a bag. All sharps, contaminated or not, must go into some kind of puncture-proof, leak-proof container. All sharps contaminated with recombinant or synthetic nucleic acid molecules must go into a **RED** sharps container. Close and process for decontamination when the container is 2/3 full. Red sharps containers can be autoclaved in-house or picked by EHS for disposal by MSU's contracted medical waste vendor.

Untreated Waste: Untreated waste for vendor removal may be picked up by the hazardous waste officer in the Environmental Health & Safety office. Please submit a request through the EHS web site.

Liquid Disinfection: Liquid disinfectants are most often used for surface treatment and, at sufficient concentration, as sterilants of liquid waste before final disposal in the sanitary sewage system. Some liquids may be chemically decontaminated. Note however, that complete reliance should not be placed on liquid disinfection if the end result must be sterility.

Disposal of Animal Waste Including Animals: If an animal that is covered by Appendix Q of the *NIH Guidelines* is euthanized or dies, the carcass will 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. A permanent record must be maintained of the experimental use and disposal of each animal or group of animals.

Disposal of Transgenic Plants and Associated Microorganisms/Small Animals: Experimental organisms will be rendered biologically inactive by appropriate methods before disposal outside of the facility. NOTE: Decontamination of run-off water is not necessarily required. If part of the facility (e.g. 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.

5. Emergency Response Plans for Research Involving Recombinant or Synthetic Nucleic Acids

Each laboratory should have plans covering the response to accidental spills and personnel contamination from research with recombinant or synthetic nucleic acid molecules. General guidance is provided below.

1. Accidental spills (Loss of Containment)

- a. An overt biological spill that occurs in the open laboratory may create a serious problem. Every effort should be taken to avoid such occurrences. A spill poses less of a problem if it occurs in a Biological Safety Cabinet (BSC) provided splattering to the outside of the cabinet does not occur.

Direct application of concentrated liquid disinfectant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work zone, but gaseous sterilants will be required to disinfect the interior sections of the cabinet. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

b. Spill in a Biological Safety Cabinet

A spill that is confined to the interior of the BSC should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet. Spray or wipe walls, work surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity, which will help clean the surfaces by removing both dirt and microorganisms. A suitable disinfectant is a 3% solution of an iodophor such as Wescodyne or a 1 to 100 dilution of a household bleach (e.g. Clorox) with 0.7% nonionic detergent. The operator should wear gloves during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container. This disinfectant, gloves, wiping cloth and sponges should be discarded into an autoclave pan and autoclaved. This procedure will not disinfect the filters, blower, air ducts or other interior parts of the cabinet. If the entire interior of the cabinet is to be sterilized, gas decontamination by a contractor is required.

c. Spill in the Open Laboratory

If potentially hazardous biological material is spilled in the laboratory, the first essential is to avoid inhaling any airborne material by holding the breath and leaving the laboratory. Warn others in the area and go directly to a wash or change room area. If clothing is known or suspected to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially

contaminated areas as well as the arms, face and hands. Shower if facilities are available. Reentry into the laboratory should be delayed for a period of 30 minutes to allow reduction of the aerosol generated by the spill.

Advance preparation for management of a spill is essential. A "Spill kit," including leak-proof containers, forceps, paper towels, sponges, disinfectant, respirators, and rubber gloves, should be readily available.

Protective clothing should be worn when entering the laboratory to clean the spill area. Rubber gloves, protective footwear, an outer garment and a respirator should be worn. If the spill was on the floor, do not use a surgical gown that may trail on the floor when bending down. Take the "spill kit" into the laboratory room, place a discard container near the spill, and transfer large fragments of material into it; replace the cover. Using a 1:10 dilution (10%) of household bleach (~5000 ppm of available chlorine), an iodophor solution containing 3200 ppm iodine, or other appropriate disinfectant, carefully pour the disinfectant around and into the visible spill. (These concentrations of disinfectants are higher than those normally employed in the laboratory because the volume of spill may significantly reduce the concentration of active ingredient in the disinfectant.) Avoid splashing. Allow 15 minutes' contact time. Use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard towels into a discard container as they are used. Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant. Place the discard container and other materials in an autoclave and sterilize. Remove shoe covers, outer clothing, respirator and gloves and sterilize by autoclaving. Wash hands, arms and face or, if available, shower.

2. Personnel Contamination

Remove contaminated clothing. Gently wash exposed area with soap and water for at least 1 minute. If eye exposure occurs, use eye wash per instructions. Flush for approximately 5 minutes. **Obtain medical attention if necessary.** If exposure results in a significant illness, contact the NIH OBA as described in part 5.

3. Types of Reportable Accidents

The following incidents involving recombinant/synthetic nucleic acids must be reported to NIH OBA within **30 days**:

- Any significant problem
- Any significant violation of the *NIH Guidelines*
- Any significant research related accident or illness

Certain accidents must be reported **immediately** and include:

- Spills/splashes in BSL-2 lab resulting in overt exposure
- Spills/accidents in BSL-4 resulting in overt or potential exposure

Reviewed by:

Biological Safety Officer

Date

Approved:

IBC Chair

Date

Appendix

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 or Synthetic Nucleic Acid 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 or Synthetic Nucleic Acid Molecule 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 or Synthetic Nucleic Acid Molecule 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 or synthetic nucleic acid molecules 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 or synthetic nucleic acid molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecule either via survival of the organisms or via transmission of the recombinant or

synthetic nucleic acid molecule 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).

Q. Transgenic 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 or synthetic nucleic acid molecules, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecules 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 or synthetic nucleic acid molecules or a recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecules. 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules. 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 or synthetic nucleic acid 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.

P. Transgenic Plants

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules. 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.