

Biosafety Level 3 – Plants (BL3-P)
***NIH Guidelines for Research Involving Recombinant or
Synthetic Nucleic Acid Molecules***
(Appendix L-II-C) – April 2024



Biosafety Level 3 - Plants (BL3-P)

Appendix L-II-C-1. Standard Practices (BL3-P)

a. Greenhouse Access (BL3-P)

- 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.
- 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.

b. Records (BL3-P)

- 1) A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
- 2) A record shall be kept of experiments currently in progress in the greenhouse facility.
- 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 OSP, and other appropriate authorities immediately (if applicable). Reports to the NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov (Contact information: 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax)). Contact information is also available on the OSP website (www.osp.od.nih.gov). Documentation of any such accident shall be prepared and maintained.

c. Decontamination and Inactivation (BL3-P)

- 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.

d. Control of Undesired Species and Motile Macroorganisms (BL3-P)

- 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.
- 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.

e. Concurrent Experiments Conducted in the Greenhouse (BL3-P)

- 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.

f. Signs (BL3-P)

- 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.
- 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.
- 3) If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

g. Transfer of Materials (BL3-P)

- 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.

h. Greenhouse Practices Manual (BL3-P)

- 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.

i. Protective Clothing (BL3-P)

- 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.
- 2) Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

j. Other (BL3-P)

- 1) Personnel are required to thoroughly wash their hands upon exiting the greenhouse.
- 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 L-II-C-2. Facilities (BL3-P)

a. Definitions (BL3-P)

- 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.
- 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.

b. Greenhouse Design (BL3-P)

- 1) The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.
- 2) Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).
- 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.
- 4) The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.
- 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.

- 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.
- 7) The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

c. Autoclaves (BL3-P)

- 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.

d. Supply and Exhaust Air Ventilation Systems (BL3-P)

- 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.
- 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.

e. Other (BL3-P)

- 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.
- 2) Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.