SWOSU BioSafety Guidelines

(Approved by the Institutional BioSafety Committee (IBC) of Southwestern Oklahoma State University on February 6, 2003.)

The IBC is appointed to review research proposals involving the use of potentially infectious organisms or recombinant DNA for proper safeguards to protect health of the employee and the environment. Membership of the Committee is specified in the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules.

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I. Introduction

Most BioSafety guidelines presented here have been excerpted from "BioSafety in Microbiological and Biomedical Laboratories", 3rd Edition, CDC/NIH and "Guidelines for Research Involving Recombinant DNA Molecules", 2002. To aid in appropriately classifying risk factors for all biological reagents and protocols in your research activity, see the Appendices below contained within the document "Guidelines for Research Involving Recombinant DNA Molecules". This document may be found in its entirety at http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html or by request from Sponsored Programs. All sectional references herein refer to this NIH document.

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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.

Four BioSafety Levels (BSLs) are described by NIH. BSL 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 and is to be adopted when using Risk Group 4 agents, whereas Biosafety Level 1 the least stringent and is to be adopted when using Risk Group 1 agents.

II. Biosafety Policies for Infectious Agents

Effective biosafety 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 facilities that provide physical barriers that are applied in varying degrees according to the estimated biohazard. The following summary table is provided by the Office of Health and Safety of the CDC:

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease. Hazards relate to percutaneous injury, ingestion, and mucous membrane exposure	BSL-1 practice plus: Limited access Biohazard warning signs "Sharps" precautions BioSafety manual defining any needed waste decontamination or medical surveillance policies	Class I or II BioSafety Containment (BSC) devices used for all manipulations of agents that cause splashed or aerosols of infectious materials Laboratory coats Gloves Face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Class I or II BSC devices used for all open manipulations of agents Protective lab clothing Gloves Respiratory protection as needed	BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon systems

The following policies are consistent with those recommended by NIH and must be followed when conducting biologically related research on the SWOSU campus with biological agents falling under the guidelines of BioSafety Levels 1 or 2. ***Biological activities or agents falling under the guidelines of BioSafety Levels 3 or 4 will NOT be permitted on the SWOSU campus.*** As such, descriptors for containment of BSL3 and BSL4 agents are not provided below.

A. Biosafety Level 1 (BSL1)

BSL1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to lab personnel and the environment. The lab is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Lab personnel have specific training in the procedures conducted in the lab and are supervised by a scientist with general training in the related science.

1. Standard Microbiological Practices (BSL1)
    a. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
    b. Work surfaces are decontaminated once a day and after any spill of viable material.
    c. All contaminated liquid or solid wastes are decontaminated before disposal.
    d. Mechanical pipetting devices are used; mouth pipetting is prohibited.
    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.
    f. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.
    g. All procedures are performed carefully to minimize the creation of aerosols.
    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.

2. Special Practices (BSL1)
    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 lab.

3. Containment Equipment (BSL1)
    a. Special containment equipment is generally not required for manipulations of agents assigned to BSL1.

4. Laboratory Facilities (BSL1)
    a. The laboratory is designed so that it can be easily cleaned.
    b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
    c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
    d. Each laboratory contains a sink for hand washing.

B. Biosafety Level 2 (BSL2)

BSL2 is similar to BSL1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL1 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; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

1. Standard Microbiological Practices (BSL2)

    a. Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.
    b. Work surfaces are decontaminated at least once a day and after any spill of viable material.
    c. All contaminated liquid or solid wastes are decontaminated before disposal.
    d. Mechanical pipetting devices are used; mouth pipetting is prohibited.
    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.
    f. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.
    g. All procedures are performed carefully to minimize the creation of aerosols.
    h. Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

2. Special Practices (BSL2)

    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.
    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.
    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.
    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.
    e. 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.
    f. Animals not involved in the work being performed are not permitted in the laboratory.
    g. 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.
    h. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

i. 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.
j. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the IBC.
k. 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.
l. A biosafety manual is prepared or adopted by the Principal Investigator. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

3. Containment Equipment (BSL2)

   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: (a) 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. (b) 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.

4. Laboratory Facilities (BSL2)

   a. The laboratory is designed so that it can be easily cleaned.
   b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
   c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
   d. Each laboratory contains a sink for hand washing.
   e. If the laboratory has windows that open, they are fitted with fly screens.
   f. An autoclave for decontaminating laboratory wastes is available.

III. BioSafety Policies Relating to Whole Plant Research

"Plants" in all discussions shall include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species. All general biosafety policies for infectious agents described above also apply to whole plant research. However, there are specific BioSafety Levels for Plants (BSL-P) for which the principal purpose of 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. 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.

A. BioSafety Level 1 for Plants (BSL1-P)

1. Standard Practices (BSL1-P)

    a. Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director when experiments are in progress.
    b. 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.
    c. A record shall be kept of experiments currently in progress in the greenhouse facility.
    d. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
    e. 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.
    f. 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.
    g. 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.

2. Facilities (BSL1-P)

    a. 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.
    b. 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.
    c. The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
    d. 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.

B. BioSafety Level 2 for Plants (BSL2-P)

1. Standard Practices (BSL2-P)

    a. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
    b. A record shall be kept of experiments currently in progress in the greenhouse facility.
    c. The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to Institutional Biosafety Committee. Documentation of any such accident shall be prepared and maintained.
    d. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
    e. 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.
    f. 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.
    g. 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.
    h. 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.
    i. 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.
    j. 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.
    k. If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.
    l. 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.
    m. 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.

2. Facilities (BSL2-P)

    a. 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.
    b. 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).
    c. An autoclave shall be available for the treatment of contaminated greenhouse materials.
    d. 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.

IV. BioSafety Policies Relating to Whole Animal Research

Experiments involving recombinant DNA or infectious agents used in conjunction with experimental animals, or animals in which the genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) or viable recombinant DNA-modified microorganisms tested on whole animals, must be reviewed and approved by the Institutional Biosafety Committee (IBC). Procedures similar to those for laboratory manipulation of microorganisms are appropriate when animals are used in research protocols. Special considerations for potential hazards caused by the animal, such as scratching, biting, creation of aerosols and waste products must be incorporated in procedures to protect animal handlers, investigators and the environment.

As with microorganisms used in the laboratory, there are four animal biosafety levels (ABSL), 1 through 4, dependent on the Risk Group classification of the recombinant DNA or infectious agents in use. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source containing less than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of ABSL 1. 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 ABSL 1. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection.

A. BioSafety Level 1 for Animals (BSL1-N)

1. Standard Practices (BSL1-N)
    a. The containment area shall be locked.
    b. Access to the containment area shall be limited or restricted when experimental animals are being held.
    c. The containment area shall be patrolled or monitored at frequent intervals.
    d. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
    e. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in animal rooms. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield.
    f. Work surfaces are decontaminated after use or after any spill of viable materials.
    g. Doors to animal rooms open inward, are self-closing and are kept closed when experimental animals are present.
    h.. All wastes from the animal room are appropriately decontaminated, preferably by autoclaving, before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leak-proof, covered containers.
    i. 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.
    j. 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.
    k. The containment area shall be in accordance with state and Federal laws and animal care requirements.

2. Special Practices (BSL1-N)

    a. The laboratory or animal facility director limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room.
    b. The laboratory or animal facility director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific requirements (e.g., immunization) may enter the animal room.
    c. Wearing laboratory coats, gowns, or uniforms in the animal facility is recommended. It is further recommended that laboratory coats worn in the animal facility not be worn in other areas.
    d. Procedures are developed by the principal investigator to address special hazards and personnel are advised of these special procedures.

3. Animal Facilities (BSL1-N)

    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.
    b. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
    c. A hand-washing sink is available.
    d. If the animal facility has windows that open, they are fitted with a fly screen.
    e. Exhaust air is discharged to the outside without being recirculated to other rooms, and it is recommended, but not required, that the direction of airflow in the animal facility is inward.

B. BioSafety Level 2 for Animals (BSL2-N)

1. Standard Practices (BSL2-N)

    Identical to those existing for BSL1-N

2. Special Practices (BSL2-N)

    a. Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.
    b. 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.
    c. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard.
    d. 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.
    e. 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.
    f. 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.
    g. 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 IBC. Medical evaluation, surveillance, and treatment shall be

provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

h. 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.

i. 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.

j. 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.

k. 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.

l. 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.

m. Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

n. 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.

3. Animal Facilities (BSL2-N)

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.

b. Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

c. The animal containment area shall be designed so that it can be easily cleaned.

d. Windows that open shall be fitted with fly screens.

e. An autoclave shall be available for decontamination of laboratory wastes.

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.

V. Emergency Procedures

Safety is an intrinsic part of each laboratory operation; work is planned so that exposure to potentially hazardous material does not occur. In spite of this, accidents do occur. These may involve spills of potentially hazardous agents in the laboratory or failure of equipment and facility safeguards that may place the laboratory worker at higher risk of exposure. Likelihood of severe injury or infection can be significantly reduced if emergency plans are established and the elements of the plans are known by laboratory workers.

It is not possible to recommend a single plan of action that would be applicable in all situations. The following basic principles, however, may be useful in developing specific procedures for dealing with an accidental spill of potentially infectious material in the laboratory.

- Get everyone out of the affected area
- Notify Campus Safety Officer (3103), Campus Police (3111), or Off-Campus Police (9911)
- Do not reenter until the extent of the hazard is determined
- Determine the need to treat persons exposed to the hazardous agent
- Decontaminate the affected area

For emergencies involving failure of equipment or facility safeguards, the most important action should be to stop work with the hazardous agent and safely contain the material until corrective action has been taken. In cases of serious injury or sudden illness, the principal investigator or laboratory supervisor should determine whether to override containment. Emergency response personnel should also be alerted to the possibility of potentially infectious agents.

General procedures for situations involving laboratory spills follow. Where a "spill kit" is indicated, this consists of a combination of personal protective equipment, disinfectants appropriate for the microorganism in use, absorbent material (paper towels), forceps, waste containers and warning signs.

A. Laboratory Spills

These are situations where a culture, specimen or container with microorganisms or blood or other potentially infectious material has breached its containment on a bench, in a biological safety cabinet, on the floor, or in an animal cage. This could be associated with manipulations in a centrifuge, grinder, or shaker or simply losing control of the container. The primary step in any loss of control incident is to contain the material to prevent its spread.

1. Spill in a Biological Safety Cabinet

   A spill that is confined in a biological safety cabinet presents minimal hazard to personnel in the area. However, chemical disinfection procedures should be initiated immediately to prevent escape of contaminants from the cabinet or cross contamination of items within the cabinet. Wipe or spray walls, work surface and equipment with a disinfectant appropriate for the type of organism being used. A disinfectant with a detergent will help clean the surfaces by removing both dirt and the microorganism. A suitable disinfectant is 70% ethyl alcohol, a 3% solution of an iodophor such as Wescodyne or a 1 to 10 dilution of household bleach. The operator should wear gloves during the disinfecting procedure. Use sufficient disinfectant solution to ensure that drain pans and catch basins below the work surface are exposed to the disinfectant. Wipe the catch basin and drain the disinfectant into a container. The disinfectant, gloves, wiping clothes and sponges should be

discarded into an autoclave bag and sterilized. This procedure does not disinfect the filters, blower, air ducts or other interior parts of the cabinet.

2. Spill in the Laboratory

   If potentially infectious 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 move a safe distance away from the area. If outer clothing, such as a lab coat is known or suspected of being contaminated, remove it with care, folding inward as it is removed. Wash all potentially contaminated areas of the skin thoroughly. Delay reentry into the lab for about 30 minutes to allow reduction of aerosols generated by the spill. Advance preparation for management of a spill is essential. A "spill kit" should be readily available.

   Protective clothing should be worn when entering the laboratory to clean the spill area. Rubber gloves, autoclavable or disposable shoe covers, an outer garment and 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 waste container near the spill, and transfer large fragments of material into it then replace the cover. Using a sodium hypochlorite solution containing 5000 ppm (1:10 dilution of household bleach) available chlorine, or other appropriate disinfectant, carefully pour or spray 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 at least 15 minutes contact time then use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard towels into a biohazard waste container for either autoclaving or disposal via commercial vendor. Remove shoe covers, outer protective clothing, respirator and gloves and either disinfect or dispose of these items.

VI. Responsibilities of the Principal Investigator

Those faculty members conducting independent research involving recombinant DNA and/or infectious agents assume the following responsibilities to the IBC:

A. General Responsibilities

1. Initiate or modify no recombinant DNA research or research involving infectious agents until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee.
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 significant problems, violations of the BioSafety Guidelines, or any significant research related accidents and illnesses to the IBC.
4. Report any new information bearing on the BioSafety Guidelines to the Institutional Biosafety Committee.
5. Be adequately trained in good microbiological techniques.
6. Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination.

7. Comply with shipping requirements for recombinant DNA molecules (see Appendix H, Shipment, for shipping requirements and the Laboratory Safety Monograph for technical recommendations).

B. Submissions by the Principal Investigator to the Institutional Biosafety Committee

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 a summary of laboratory protocols and infectious agents and any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the Institutional Biosafety Committee for review and approval or disapproval. The "BioSafety Compliance Form for Biochemical / Biological Research on the SWOSU Campus" (BSC.doc) is to be used by any faculty engaged in research using recombinant DNA and / or potentially infectious agents.
4. Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

C. Responsibilities of the Principal Investigator Prior to Initiating Research

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.
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

D. Responsibilities of the Principal Investigator During the Conduct of the Research

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 IBC.
3. Correct work errors and conditions that may result in the release of recombinant DNA materials.
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).

Adopted: February, 2004
The *SWOSU BioSafety Guidelines* have been approved by the SWOSU BioSafety Standing Faculty Committee and SWOSU Provost's Office.