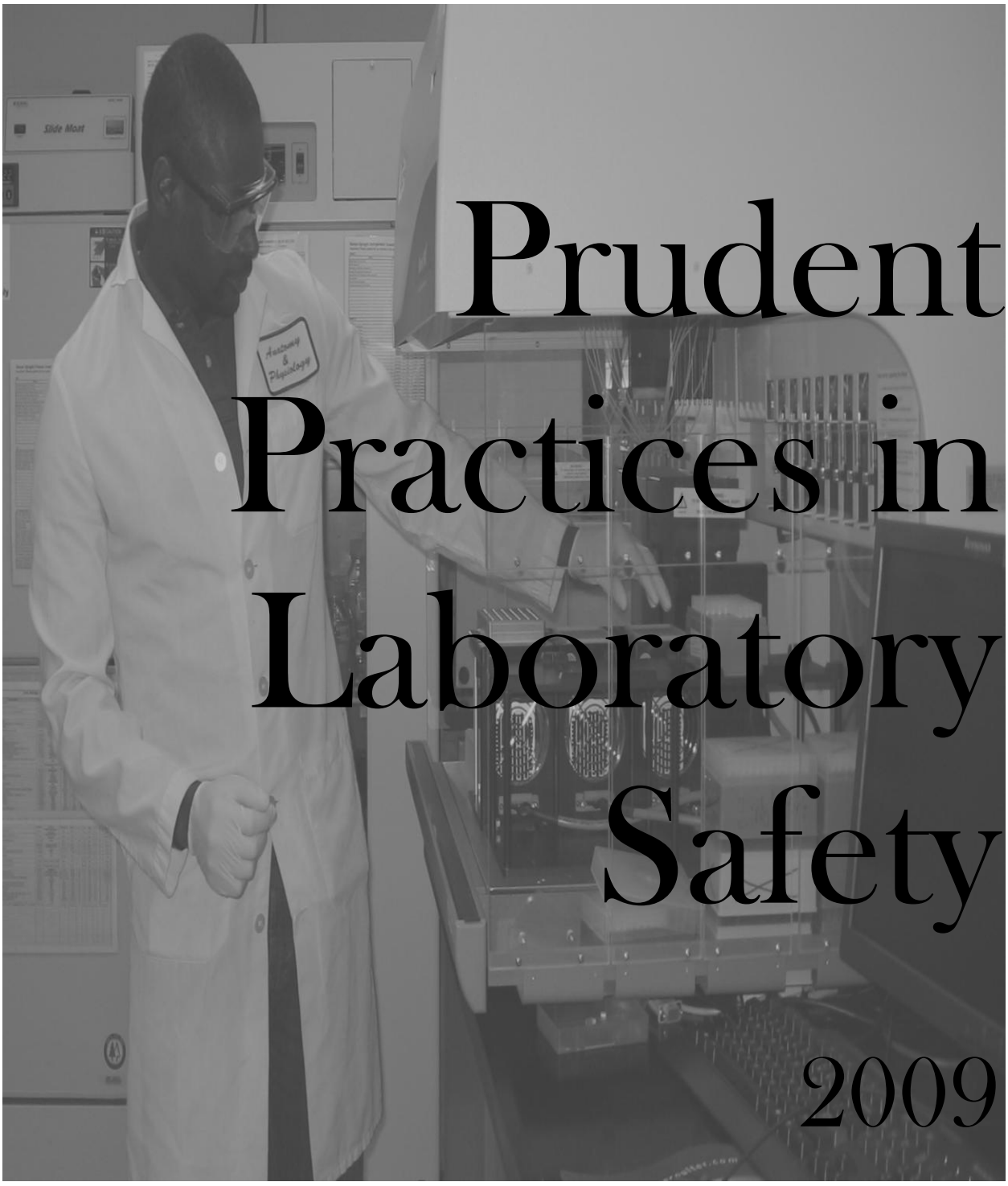

Prudent Practices in Laboratory Safety

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Prudent Practices in Laboratory Safety
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Thanks to Dr. Kalidou Ndiaye, Assistant Scientist with Dr. Daniel Marcus,
for allowing his picture on the cover of this publication.

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I. INTRODUCTION

To improve overall campus and laboratory safety, the President of Kansas State University annually appoints a Campus Environmental Health and Safety Committee. The committee hopes with this revision of the Good Laboratory Safety Practices manual to describe the more common hazards in science laboratories in an effort to instill safety awareness in students, faculty, and staff.

This manual describes general standards and guidelines that should be followed to keep laboratory accidents to a minimum. The information has been collected from the most current Federal and State guidelines as well as current chemical safety guides. Investigators should use these guidelines to complete their work with a minimum of injury, lost time, and expense. Students should initiate habits and practices that will insure their safety and take safety awareness into their future workplace.

II. IN CASE OF EMERGENCY

A. Emergency Telephone Numbers

Phone Number	Agency
911 or 2-6400	ANY EMERGENCY SUCH AS FIRE, CHEMICAL RELEASE, AMBULANCE, AND POLICE - Kansas State University Police Department
2-6544	STUDENT CARE ONLY - Lafene Health Center
2-5856	ALL SAFETY ISSUES - Division of Public Safety
 2-3233 2-5640	ANY EMERGENCY INVOLVING ANIMALS Dr. Jerry Jaax, University Veterinarian Dr. Kerry Taylor, University Veterinarian
9-776-3322	Mercy Hospital at College – Emergency Room
9-1-800-332-6633	Mid-America Poison Control Center

B. Emergency Plans and Procedures

1. In Case of Fire.

   a. The Division of Public Safety is responsible for maintaining and checking all fire alarm systems, smoke alarms, fire extinguishers and other fire suppression systems. In case of any type of medical or fire emergency call 911.

   b. All employees must be trained on how to use a fire extinguisher. Faculty should make every effort to acquaint students in class of the locations of emergency equipment and fire exits. Training for fire extinguisher use is available for employees and students from the Division of Public Safety, call 2-5856 to arrange training.

   c. In the event of a fire, pull the fire alarm as you leave the area and call 911 to report the location of the fire. Fire alarms are not all connected to the Manhattan Fire Department. In the event of a fire alarm, all persons must proceed to the nearest exit

for evacuation. Instructors should announce the location of the nearest exit at the beginning of the class each semester. All evacuees should meet in a location away from the building and out of the way of the emergency vehicles. All efforts should be made to aid physically disabled individuals to a safe location and to advise emergency rescue personnel.

2. In Case of Tornado or Other Severe Weather.

a. All building occupants should know where to go in case of a tornado or severe weather. In the event of a tornado, the sirens will sound a steady three-minute blast when there is need to take cover. Sirens are intended to alert personnel outside the building. For those inside buildings, an alert will be sent to personnel in every building via the telephone. Each department head should prepare a plan on how personnel will be advised of the weather emergency.

b. In general, you should:

(1) Get and stay indoors during the storm.

(2) Go to the interior hallways on the lowest level of the building.

(3) Stay away from windows, doors, outside walls and protect your head.

(4) Listen for improved weather conditions on a local radio or television station.

c. The University Fire Marshal has placed tornado refuge signs near each building exit. By order of the State Fire Marshal (K.A.R. 22-18-2), these signs must be posted and must not be removed. For questions or replacement sign(s), call 2-5856.

d. After the severe weather emergency has passed, faculty or staff should notify the proper emergency personnel of any damages or injuries by calling 911. All university related injuries or illnesses must be reported through Accidental Injury Forms, located in the departmental office, as per the Policy and Procedures Manual.

3. In Case of Chemical Spills or Releases.

a. Chemical spills come in two sizes – small and large. A small spill means 500 ml or less of a moderate or less hazard chemical; in general, a spill covering less than a nine inch diameter of any hazardous material is considered a small spill. Any spill greater than a small spill is considered a large spill. No matter what size spill occurs, report the spill to the Division of Public Safety by calling 911.

b. In the event of chemical spills or releases, all efforts should be made to contain the spill using absorbents. Alert all personnel in adjoining rooms to turn on any fume hoods and leave the area. Close any doors to the hallway on the way out. All affected personnel should meet at a safe location and await emergency response personnel. While waiting for emergency response personnel, retrieve a copy of all relevant Material Safety Data Sheets.

c. Department personnel should clean up small chemical spills; the Division of Public Safety will assist if needed. Faculty and staff working with chemicals must be trained for spill cleanup. It is recommended that each department develop a list of personnel who can assist with cleanup. The Department of Environmental Health and Safety has some materials available for spill clean-up, however, departments with a high

probability for spills should purchase their own materials. All employees who work with chemicals must be trained on proper spill control, clean-up, and disposal.

d. The Division of Public Safety must be notified of any size spill immediately by calling 911 to report the spill. University Police will notify the appropriate personnel. The caller should provide information about the spill, specifically what chemical spilled and the quantity spilled.

e. Each laboratory must have access to the necessary materials to clean up chemical spills. An extensive list of materials for a hazardous spill response cart is contained in Appendix C. These materials include but are not limited to:

(1) Protective clothing.
(2) Chemical absorbent material.
(3) Acid/base neutralization chemicals.
(4) Polypropylene squeegee.
(5) Drain stopper.
(6) Polypropylene shovel.
(7) Polypropylene pan and broom.
(8) Barricade or caution signs.

f. Some chemical releases must be reported to the U.S. Environmental Protection Agency (EPA) or other government agencies by the Division of Public Safety.

4. In Case of Radioactive Material Spills or Releases. Exposures to radioactive materials must be reported immediately to the Radiation Safety Officer. Call 911 to report the spill. Users of radioactive materials must be familiar with the Radiation Safety Manual concerning accident procedures.

5. Faculty and staff should be familiar with their surroundings.

a. Faculty and staff should be familiar with the hazardous materials and hazardous situations that exist in their building. New employees must be trained in the use of hazardous materials and situations within the first few weeks on the job. It is recommended that each department develop a list of hazardous materials and hazardous situations such as, "flammable chemicals – 237 King Hall." List those areas where there is limited or restricted access.

b. Faculty and staff should be familiar with the location and use of emergency fire equipment. This includes the location of fire extinguishers, fire alarm pull stations, first aid supplies, fire blankets and emergency showers. It is recommended that each department develop a list of emergency equipment and its location.

c. Faculty and staff should be familiar with the location and use of emergency chemical spill equipment. This includes the location of chemical spill kits, personal protective equipment and first aid supplies. It is recommended that each department develop a list of emergency equipment and its location.

III. RESPONSIBILITIES

A. The Division of Public Safety is responsible for the safety of the students, faculty and staff in research and teaching laboratories. In carrying out this charge, the Division of Public Safety is responsible for development and implementation of campus safety standards.

Division personnel will also serve as consultants to assist departments with environmental health and safety issues.

B. The Dean of each College is responsible for appointing a College Environmental Health & Safety Committee and to ensure that personnel adhere to current safety standards.

C. The Department Head is responsible for appointing a Department Safety Coordinator and keeping the Division of Public Safety informed of personnel changes.

D. The Department Safety Coordinator is responsible for chemical safety, chemical spill notification, emergency contingency plans and Hazard Communication training.

E. The Principal Investigator is responsible for the training of employees and students in proper procedures, following a Chemical Hygiene Program, and for seeing that safe practices are followed. This individual is responsible for general laboratory safety, hazardous waste disposal and liaison with the Division of Public Safety.

F. Employees and students working in or using the laboratory facilities in the course of their employment or studies are responsible for knowing and following all safety procedures.

IV. GENERAL LABORATORY SAFETY PRINCIPLES

A. Know your materials.

1. Minimize all chemical exposures.

2. Approach all chemicals as hazardous and use caution.

3. Avoid underestimating the risk. Assume that any mixture will be more toxic than its most toxic component and that all substances of unknown toxicity are highly toxic.

4. Observe the Threshold Limit Values (TLVs). The TLVs of the American Conference of Governmental Industrial Hygienists (ACGIH) should not be exceeded.

B. Provide adequate ventilation. Use fume hoods and other ventilation devices properly to prevent exposure to airborne substances.

C. Follow safe practices and use Standard Operating Procedures (SOPs).

D. Know emergency procedures and location of safety equipment.

E. Institute a Chemical Hygiene Program. These guidelines set forth procedures, personal protective equipment use, and work practices that protect the health of personnel subjected to hazardous materials.

V. LABORATORY SECURITY

A. Laboratory security is an integral part of an effective laboratory safety program. Follow these procedures to ensure a secure working environment.

1. Keep laboratory doors locked when unoccupied.

2. Keep stocks of organisms locked during off hours or when the laboratory is unoccupied.

3. Keep an accurate record of chemicals, stocks, cultures, project materials, growth media, and those items that support project activities.

4. Notify University Police (2-6412) if materials are missing from the laboratory.

5. Inspect all packages arriving at the work area.

6. When research is completed for the day, ensure that chemicals and biological materials have been properly stored and secured.

7. Ask strangers (someone you do not recognize as a co-worker or support staff) for identification. If they are not authorized to enter, ask them to leave.

B. Keep high hazard materials under lock and key. High hazard materials include:

1. Radioisotopes

2. Carcinogens

3. Select Agents

4. Narcotics

C. Maintain a catalog for receiving, using, and disposing of high hazard materials.

D. For laboratories using Select Agents. There are a number of additional specific regulations that must be followed, see section VIII.G.

VI. CHEMICAL HYGIENE PROGRAM

A. The complete Chemical Hygiene Program can be found at: http://www.k-state.edu/safety. Personnel are encouraged to review the entire document to fully understand the Lab Standard.

B. This chemical hygiene program applies to all colleges on Kansas State University campuses that engage in the laboratory use of hazardous chemicals. This program does not apply in those laboratory uses of hazardous chemicals which provide no potential for employee exposure. Examples are: the use of "Dip and Read" test where a reagent strip is dipped into the specimen and the results are interpreted by comparing the color reaction to a color chart; and the use of completely self-contained commercially prepared kits.

C. Responsibilities. The Division of Public Safety will designate an employee as the university's Chemical Hygiene Officer. The Chemical Hygiene Officer will assist if requested to provide chemical exposure monitoring.

D. Employee Exposure Monitoring. If there is reason to believe that exposure levels in the workplace routinely exceed the TLV, the Department Head will initiate monitoring (initial monitoring). If initial monitoring discloses employee exposure above the TLV, the Department Head will initiate periodic monitoring. Affected employees will be notified of the monitoring results. Contact the Division of Public Safety for monitoring. Cost for such monitoring is the responsibility of the requesting department.

E. Components of the Chemical Hygiene Plan.

1. Comply with University Laboratory Safety Rules as published in the Division of Public Safety policies:

- Prudent Practices in Laboratory Safety
- Safety with Chemical Carcinogens in Research and Teaching

- Radiation Safety Manual
- Laser Safety Manual
- Hazard Communication Program
- Chemical Hygiene Program
- Any other rules set as policy by the Campus Environmental Health and Safety Committee.

2. Develop written Standard Operating Procedures in laboratories.

3. Train employees to understand the hazards in the laboratory. Training should include appropriate work practices, emergency procedures, and use of personal protective equipment.

4. Medical consultation and examinations whenever:

a. an employee develops signs or symptoms associated with a hazardous chemical to which the employee may have been exposed in the laboratory;

b. environmental monitoring reveals an exposure level above the TLV;

c. a chemical spill, leak, explosion or other release takes place which results in a hazardous exposure; or

d. when respirator use is necessary to maintain exposure below the TLV. Every effort will be made by the Department Head to provide the necessary engineering controls to relieve the need for respirators.

VII. RECOMMENDED SAFE LABORATORY PRACTICES

A. General Safety Rules.

1. Do not work alone in the laboratory.

2. Clean up all spills and leaks promptly. Spill kits should be purchased and used to assist in clean up operations.

3. Do not store or consume food and beverages in laboratories or near chemicals.

4. Do not smoke in laboratories.

5. Avoid smelling or tasting chemicals.

6. Avoid using damaged glassware. Broken glassware should be discarded in sealed boxes, not in the regular trash receptacles.

7. Used needles and syringes, razor blades, Pasteur pipettes and other sharps should be placed in special "sharps" containers available from the Division of Public Safety.

8. Shield or wrap Dewar flasks (vacuum glass apparatus).

9. Wash exposed skin well before leaving the laboratory.

10. Horseplay, practical jokes, or other acts of carelessness are prohibited.

11. Oral pipetting or mouth suctioning of hazardous, caustic, toxic, radioactive, cancer causing, or biological specimens is prohibited.

12. Personal clothing.

a. Confine long hair and loose clothing.

b. Wear shoes at all times in the laboratory. Sandals, flip-flops, perforated shoes, open-toed shoes, or canvas sneakers are prohibited in the laboratory.

13. Personal housekeeping.

a. Each individual is responsible for keeping the work area clean and uncluttered.

b. Chemicals and equipment should be clearly and correctly labeled as well as properly stored.

c. Clean up work area upon completion of a procedure, at least at the end of each day.

14. Sink traps and floor drain traps should be kept filled with water at all times to prevent escape of odors to other building areas.

15. Each worker should use proper personal protection that includes, as a minimum, safety glasses, chemical resistant gloves, and a laboratory coat or apron.

16. Appropriate warning signs should be posted near any dangerous equipment, reaction, or condition.

17. Interior connecting doors between laboratories should be unobstructed and unlocked at all times.

18. Adequate, skid-proof footstools and stepladders should be used for reaching upper shelves. Do not stand on chairs or other easily movable objects.

19. All equipment should be inspected for defects prior to use.

20. Gas, air, and vacuum services should be turned off at the bench service valve when services are not in use.

21. Vigilance - be alert to unsafe conditions and correct them when detected.

B. Personal Protective Equipment.

1. Eye protection. State law (K.S.A. 72.5207) requires that every student and teacher participating in vocational, technical or industrial arts shops or laboratories must wear eye protective devices suitable to protect against the hazard.

a. Eye protection is required in all instances in chemical, physical or microbiological laboratories where, aerosols, splashes, sparks dust, metals, etc., may be created. This includes all teaching areas.

b. A face shield may also be worn for added protection, as appropriate. Face shields alone are not adequate.

c. Proper eye protection must be worn by all students and instructors in chemical laboratories. Non-vented or protected-vent goggles are preferred for potential chemical splashes.

d. Caution should be used when wearing contact lenses in laboratories. Do not wear contact lenses in laboratories where chemicals are used that may affect the contact lens. If worn, wrap-around goggles should be used for added protection. Some

chemical vapors have a tendency to adsorb into or under contact lenses and create a hazard to the wearer. Ask your ophthalmologist or optometrist for advice concerning your contact lenses.

2. Proper gloves must be worn when working with organic solvents, corrosives, toxic materials, allergens, or pathogenic organisms. Inspect gloves before use, wash them before removal, and replace them periodically. Disposable gloves should not be reused. **Remove gloves prior to leaving the laboratory.**

3. Clothing.

a. Aprons should be worn when conducting operations where the chemicals used can cause skin irritations.

b. Laboratory coats protect street clothes and prevent "bringing home" dangerous chemicals or pathogenic organisms. Remove laboratory coats when leaving the laboratory. Change laboratory coats immediately upon significant contamination.

c. Do not wash laboratory clothing at home.

4. Wash arms and hands immediately after working with allergens, carcinogens, pathogenic organisms, or toxic chemicals. Wash exposed skin well before leaving the laboratory.

C. Emergency Equipment.

1. Emergency showers and eyewash stations. Suitable facilities for quick drenching or flushing of the body and eyes must be provided near the work area for emergency use where eyes or body of any person may be exposed to injurious, corrosive materials. The shower and/or eyewash must be located within 10 seconds of unobstructed travel.

a. Operating chains or bars must be freely accessible so the shower can be used in the event of an emergency.

b. The area beneath and in front of each safety shower and eyewash station must be kept clear and unobstructed at all times.

c. Eye and face wash facilities capable of immediate and extended (15 minutes), continuous flow must be available to laboratory users. Many laboratories have eyewash bottles available for an emergency. While these provide immediate response, they do not provide extended continuous flow. In addition, these products are dated. Check the dates and replace when past the date.

d. All plumbed eye and face washes should be flushed by laboratory users on a weekly basis by allowing the water to flow for approximately 3 minutes to remove stagnant water from the pipes.

e. Emergency showers should be tested frequently by laboratory personnel to ensure safe operation when needed.

2. Fire Extinguishers.

a. Fire extinguishers are placed in or near laboratories depending on the degree of fire hazard.

b. Fire extinguishers are generally placed 36 inches off the floor near the laboratory

door or near the fire hazard.

c. Fire extinguishers are supplied as Dry Chemical or Carbon Dioxide, depending on the fire hazard. If you need a fire extinguisher or need one moved, contact the Division of Public Safety for assistance.

d. Laboratories that require Class D (flammable solids) fire extinguishers, should consult with the Division of Public Safety. Class D fire extinguishers must be purchased by the using laboratory, but the Division of Public Safety will provide inspections and maintain records.

e. Fire extinguisher demonstration and training is provided by the Division of Public Safety, upon request.

3. First Aid Kits.

a. First aid kits should be available in each laboratory.

b. Purchasing and maintaining first aid kits is the responsibility of the laboratory.

c. Minimum requirements for first aid kits can be found at: http://www.k-state.edu/safety, including:

(1) absorbent compress

(2) adhesive bandages

(3) adhesive tape

(4) sterile pads

(5) triangular bandage

(6) individual-use antiseptic applications

(7) individual -use burn treatment applications

(8) 2 pairs medical exam gloves

(9) Do *NOT* add aspirin, acetaminophen, ibuprophen, antihistamines, cold medications, or other over the counter medications.

4. Signs and Other Door Postings.

a. Appropriate signs must be posted and maintained. If a sign becomes damaged, faded, or vandalized, it must be replaced.

b. Radiation signs are provided by the Radiation Safety Officer. All other signs are the responsibility of the laboratory.

c. Each exterior laboratory door to the corridor must be posted with current emergency information that includes the name and home phone number of the laboratory director, manager, or other responsible faculty member as well as one other responsible person.

D. Electrical and Mechanical Protection.

1. All electrical equipment and services must be grounded.

2. All electrical cords which are frayed or deteriorated must be replaced.

3. Electrical cords and instrument cables should not be located near potential heat sources, in locations where they may be subjected to wear by friction, or where they may present a shock or fire hazard.

4. Equipment electrical cords and extension cords must not be placed above ceiling tiles, through doorways or walls, or located where they will present a tripping hazard.

5. Extension cords may only be used for temporary conditions. They must not be used in place of permanent wiring. If extension cords are used, be sure the cord rating is adequate for usage and is a grounded type.

6. Only explosion-proof equipment should be used inside a fume hood when explosive vapors may be present.

7. Only refrigerators that are IM or UL listed as FLAMMABLE-STORAGE or EXPLOSION-PROOF should be used for flammable liquid storage. Flammable-storage indicates that flammable materials are isolated from sparks. Explosion-proof indicates that the entire unit is sealed and can be used in explosive atmospheres.

8. Machinery belts and pulleys must be guarded to prevent clothing or parts of the body from being caught in the belt. This can be accomplished by use of a protective shield or other protective installation.

E. Fume Hoods.

1. All laboratories must provide an environment that is safe from microbes, fumes, vapors, dusts, carcinogens and radioactive materials that may be generated during an experiment. The purpose of any fume hood is to capture the offending fume, vapor, or gas in an air stream of sufficient velocity to entrap and carry it safely outside the laboratory. At the same time, the fume hood must allow the investigator to perform experimental procedures with safety and dexterity.

2. The fume hood's face velocity is crucial in preventing hazardous vapors from reaching the breathing zone of the user. The face velocity is the exhaust velocity at the opening or sash of the fume hood. The face velocity is affected by turbulence generated by the user at the opening, equipment and supplies inside the hood, drafts from doors, windows, and air vents in the laboratory, and even people walking by the fume hood while it is operating. All of these factors must be kept at a minimum.

3. Minimum face velocities in feet per minute (fpm) at Kansas State University are:

- 80 fpm for low toxicity level materials including noxious odors, nuisance dusts, and fumes;
- 100 fpm for general laboratory use, including moderate toxicity level materials and trace quantities of radioisotopes; and
- 125-150 fpm for high toxicity level materials including certain radioisotopes, perchloric acid and carcinogens.

4. The maximum face velocity in fume hoods is 150 fpm. Face velocities exceeding 150 fpm tend to develop currents inside the fume hood that result in vapor exposure to the

user.

5. Fume hoods should be kept closed to the smallest sash opening that still allows for adequate ventilation. The sash should be kept closed except when moving materials in or out of the fume hood. To properly use a fume hood:

a. open the fume hood sash to its widest extent to place equipment or chemicals;

b. close the fume hood sash to within twelve to fourteen inches of the base to allow placement of arms for manipulation of materials; or

c. close fume hood sash to the base, slide horizontal sash to protect your face and body while allowing placement of arms for manipulation of materials.

6. Many fume hoods have moveable baffles to allow proper exhaust. The bottom baffle should be open to exhaust heavier than air vapors that tend to settle and the top baffle should be open to exhaust lighter than air vapors that tend to rise. For most general laboratory fume hoods, both baffles should be open. Keep the openings free of obstruction by apparatus or containers.

7. Do not work in a non-operating fume hood.

8. Keep all apparatus at least six inches back from the face of the hood. Placing a stripe on the bench surface is a good reminder.

9. Do not store chemicals or equipment in a fume hood.

10. Clean up any chemical spills in a fume hood when they occur.

11. Do not put your head in the hood when contaminants are being generated.

12. Do not place electrical receptacles or other spark sources inside the hood when flammable liquids or gases are present. Permanent electrical receptacles should not be placed in the hood.

13. Use an appropriate barricade if there is potential for an explosion or eruption.

14. Fume hoods are tested annually by the Division of Public Safety. Results are posted on the fume hood as well as reported to the department head. The velocity is measured at the center of square foot sections on the face of the sash with the sash open to its full extent or to the stops, if present. In addition, smoke is used to ensure that the entire face of the fume hood is exhausting out and not into the laboratory.

15. Observe static pressure gauges, velocity monitors or other operator indicators to insure that the exhaust system is properly working. In case of failure, notify your department head.

16. When Facilities repair personnel are called:

a. Do not operate a fume hood when it is being serviced;

b. Remove any chemicals or equipment from the fume hood before maintenance personnel perform servicing;

c. Wash down the interior of the fume hood with soap and water before maintenance personnel work inside the fume hood.

d. Maintenance personnel will put on personal protective equipment such as coveralls, goggles, gloves, and respirators when servicing your fume hood; and

e. When maintenance personnel are working on the roof, you may be required to discontinue fume hood operations. If you are requested to do so, do not operate fume hoods.

F. Biological Safety Cabinets (Biohazard Cabinets).

1. Biohazard cabinets are special hoods equipped with High Efficiency Particulate Air (HEPA) filter systems and designed to provide personal and environmental protection from biohazardous material or product protection from contamination. For discussion of biohazards, see section VIII.G.

2. Biohazard cabinet classes:

a. Class I - a ventilated cabinet for personal and environmental protection, with a non-re-circulated airflow away from the operator. Similar to fume hoods, except it may or may not be connected to an exhaust duct system. May be used for Level 1, 2 or 3 containment. There is no product protection.

b. Class II - a ventilated cabinet for personal, environment and product protection with an inward HEPA filtered airflow for personal protection. May be used for Level 1, 2 or 3 containment.

c. Class III - a totally enclosed, ventilated cabinet of gas-tight construction. Operations in the cabinet are completed through attached rubber gloves. The cabinet is kept under slightly negative air pressure. Supply air is HEPA filtered and exhaust is double HEPA filtered or a combination of HEPA filter and incineration. May be used for Level 4 containment.

d. Laminar Flow Clean Bench - a laminar flow cabinet for product protection. No effort is made to control aerosols generated in the work area or to protect the user. May not be used with biohazard materials, toxins or radioisotopes.

e. Avoid the use of flammable gases or solvents in biohazard cabinets. Care must be taken to ensure against the concentration of flammable or explosive gases or vapors.

f. Do not use open flames in biohazard cabinets.

g. Ultraviolet (UV) lamps are frequently used in biohazard cabinets for decontamination of the work area. Use of UV lamps will help to maintain disinfection, but should not be relied on for the sole disinfection of the work area. Do not stare directly at the lamp when on. The light must be off when working in the cabinet. *Do not operate the UV lamps when hands are in the cabinet.*

h. Biohazard cabinets should be tested and certified on an annual basis, if maintenance is needed, or after the unit is moved. The Division of Public Safety does not test or certify biohazard cabinets.

3. Proper use of a biohazard cabinet.

a. Thoroughly understand procedures and equipment required before beginning work.

b. Arrange for minimal disruptions in the work area while in use.

c. Turn off the UV lamp, if present.

d. Ensure that the sash is set at its lowest position.

e. Turn on cabinet light and blower.

f. Check the air grills for obstruction and check pressure gauge.

g. Wash hands and arms thoroughly with germicidal soap before working in the cabinet.

h. Wear proper personal protective equipment including a long sleeved laboratory coat with knit cuffs and over-the-cuff gloves, eye protection and surgical mask, if appropriate.

i. Wipe down the interior surfaces of the cabinet with 70% ethanol or other suitable disinfectant and allow to dry.

j. Load materials required for the procedure into the cabinet. Do not overload the cabinet.

k. Do not obstruct the front, side or rear air return grills.

l. Do not place large objects close together.

m. After loading the cabinet, allow several minutes to purge airborne contaminants from the work area.

n. Keep all materials at least four inches inside the sash and perform all operations as far to the rear of the work area as possible.

o. Keep clean and contaminated materials segregated. Arrange materials to minimize the movement of contaminated materials into clean areas. Keep all discarded contaminated material to the rear of the cabinet.

p. Avoid moving materials or arms through the front access during use. Avoid techniques or procedures that disrupt the airflow patterns of the cabinet.

q. In the event of a spill during use, decontaminate all objects prior to removal. Thoroughly disinfect the work area while the cabinet is still in operation.

r. Upon completion of the work, operate the cabinet for several minutes undisturbed to purge contaminants from the work area.

s. Surface decontaminate all objects and cover all trays or containers prior to removal from the cabinet.

t. Wipe down the interior surfaces of the cabinet with 70% ethanol or other suitable disinfectant and allow to dry.

u. Wash arms and hands thoroughly with germicidal soap.

v. Turn off the interior light and blower and turn on the UV light if available.

4. Working with Cytotoxic or Hazardous Drugs.

a. Work in a Class II biohazard cabinet only.

b. Operate the cabinet continuously, i.e., 24 hours per day, seven days per week.

c. Train workers in proper manipulative technique.

d. Decontaminate at least weekly or if there is a spill.

e. Certify cabinet every six months.

G. General Handling and Storage of Chemicals.

1. Know and understand the Hazard Communication (Worker Right-To-Know) Program.

2. Do not store food in refrigerators or freezers used for chemical or biological storage. Refrigerators and freezers must be labeled "NO FOOD" or "FOOD ONLY - NO CHEMICALS" depending on the intended purpose of the equipment. Labels are available from the Division of Public Safety.

3. All chemical storage containers must be labeled with the name of the contents and appropriate hazard warnings. The label must contain the full chemical name, not abbreviations or chemical formulas.

4. Chemicals must be stored based on compatibility. Chemicals should not be stored in alphabetical order but should be stored by hazard class, i.e., flammables, acids, bases, oxidizers, reactives, poisons, etc., with classes segregated from each other. Nitric acid is an oxidizing agent and should be stored away from other acids. See Appendix A.

5. Flammable and combustible liquids storage.

a. Flammable liquids must be kept in closed containers when stored in refrigerators or freezers.

b. Refrigerators, freezers and other cooling equipment used for storing flammable liquids should be rated for storing such items and be prominently labeled as such. Equipment that is IM or UL listed as *FLAMMABLE-STORAGE* or *EXPLOSION-PROOF* should be used for flammable or volatile liquid storage.

(1) Flammable-storage indicates that flammable materials are isolated from sparks.

(2) Explosion-proof indicates that the entire unit is sealed and can be used in explosive atmospheres.

c. Flammable and combustible liquids should be stored and used in approved safety containers. Safety containers have a maximum capacity of 5 gallons, have a spring-closing lid and spout cover and are designed to safely relieve internal pressure when subjected to fire exposure.

d. An outside flammable liquid storage facility must be used for storage of flammable and combustible chemicals in containers greater than 30 gallons.

e. A "Flammable Liquid" storage room, preferably one protected with automatic fire suppression equipment, should be available for interior storage of flammable chemicals no larger than 30-gallon containers.

f. UL approved "Flammable Storage" cabinets should be used for limited chemical storage in individual laboratories.

(1) Flammable storage cabinets should be properly designed and constructed

according to National Fire Protection Association (NFPA) guidelines and labeled "FLAMMABLE-KEEP FIRE AWAY."

(2) Flammable and combustible liquids are defined by their flash point:

(a) Flash point is the temperature at which a liquid will burst into flame, if an ignition source is present.

(b) Liquids with a flash point below 100°F (37.8°C) are considered a Class I flammable liquid.

(c) Liquids with a flash point at or above 100°F and below 140°F (60°C) are considered a Class II combustible liquid.

(d) Liquids with a flash point at or above 140°F and below 200°F (93°C) are considered a Class IIIA combustible liquid.

(3) Not more than 5 gallons of Class I and Class II liquids combined may be stored in a laboratory space outside of a safety cabinet unless in safety containers.

(4) Not more than 25 gallons of Class I and Class II liquids combined may be stored in laboratory space outside of a safety cabinet IF stored in safety containers.

(5) Not more than 60 gallons of Class IIIA liquids may be stored in a laboratory space outside of a safety cabinet.

(6) A maximum of 75 gallons (or 150 gallons if a sprinkler system is installed) of Class I liquids may be stored in a laboratory space combining the total of inside and outside of storage cabinets and safety containers.

(7) A maximum of 100 gallons (or 200 gallons if a sprinkler system is installed) of Class II and Class IIIA liquids may be stored in a laboratory space combining the total of inside and outside of storage cabinets and safety containers.

g. Flammable solvents, such as anhydrous diethyl ether, should be stored in an explosion-proof or flammable-storage refrigerator. A cold box may be used if explosion-proof electrical fixtures are installed.

h. Do not store oxidizers with flammable materials in refrigerators or freezers.

i. Oily rags and similar waste should be placed in metal containers with self-closing tops.

j. Operations or equipment that create ignition sources, such as open flames, hot plates and other heat elements, should be eliminated from flammable liquid areas.

k. Metal containers must be grounded when transferring solvents from one container to another. Static sparks can start solvent fires.

6. Storage quantity of all liquids generally should be limited to that required for operation of equipment, maintenance, demonstration, treatment, and/or laboratory work.

7. Materials should not be placed so as to limit use of exits, stairways, or areas normally used for the egress of people.

8. Glass containers should not be stored directly on the floor. Eliminate, when possible, storing glass containers on high shelves.

9. Reagent containers should not be stored on bench tops.

10. Laboratory benches and aisles should not be used as storage areas but should be cleaned upon completion of each experiment and at the end of each day.

11. Dispensing solvents and other hazardous chemicals should be accomplished in a fume hood or in a well ventilated area. Fume hoods should be used to confine and exhaust odoriferous, corrosive, and toxic vapors generated in a laboratory.

12. Substances with particularly noxious or toxic vapors should be double containerized and sealed or temporarily stored in a continuously operating hood.

13. All vacuum vessels, including Dewar flasks and vacuum desiccators, should be inspected for damage. Taping the vessels can reduce implosion hazards. Use personal protective equipment including explosion-proof shields as appropriate. Vacuum pumps should not be located inside closed cabinets or under low bench tops where excessive heat build-up can occur. Do not place pumps near containers of flammable solvents.

14. Flames, open elements, or exposed heat sources must never be left unattended, even for short periods of time. Keep open flames and hot plates away from flammable liquids.

15. Use equipment only for its designed purpose. Do not use faulty equipment.

16. Chemical Inventory. Maintain a current inventory of all chemicals and submit a copy to the Division of Public Safety in January of each year.

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VIII. SPECIAL LABORATORY HAZARDS

A. Special Chemical Hazards.

1. Peroxide forming chemicals.

a. Date all ethers when received and when first opened.

(1) Isopropyl ether, isoamyl ether, and anhydrous ethers should not be kept over six months.

(2) Diethyl ether and other ethers should not be kept over one year unless precautions are taken to avoid peroxide formation.

(3) The hazard is much greater in opened containers.

b. Compounds that are suspected of having very high peroxide levels, because of visual observation of unusual viscosity, crystal formation, or age should be considered extremely dangerous.

IT IS OF THE UTMOST IMPORTANCE THAT THE CONTAINER NOT BE OPENED. THE ACT OF OPENING THE CONTAINER COULD DETONATE PEROXIDE CRYSTALS UNDER THE CONTAINER CAP.

c. All work involving ethers should be done in a fume hood and adequate personal protection such as a safety shield, a face shield and goggles should be used.

2. Perchloric acid.

a. Use of hot acid or concentrations above 72% add greatly to the hazard. Acid strengths of 90-100% are very hazardous and may explode spontaneously.

b. Contact of perchloric acid with oxidizable or combustible materials, or with dehydrating or reducing agents may result in fire or explosion.

c. Spilled perchloric acid should be immediately diluted with water and, if possible, neutralized with soda ash. Dispose of by flushing down drain with a large quantity of water or soak up with paper towels. Place towels in a plastic bag; seal and place in a disposable container for flammable waste. If acid is soaked up by paper towels, the acid must be neutralized first!

d. Fuming perchloric acid must be used in a perchloric acid fume hood with wash-down capabilities. Perchloric acid fume hood systems should be washed down after each day's use.

e. Laboratory quantities may be temporarily stored in a perchloric acid fume hood but should be limited to a one pound (500 g) reagent bottle per hood.

3. Hydrofluoric Acid.

a. Before working with hydrofluoric acid (HF) make sure you are completely familiar with the HF material safety data sheet (MSDS) for the specific form of HF being used. You should have a written procedure for working with and responding to exposures to HF.

b. Personnel should be trained on the health hazards of HF and the medical emergency response procedures. Call the Division of Public Safety, 2-5856, for training information.

c. If exposure occurs, call 911 immediately. Special procedures and treatment is necessary for exposure to HF. The acid cannot be treated as would exposure to other acids. The treatment for HF exposure varies, depending on what body part was exposed and whether the exposure was to aqueous HF or anhydrous HF (gas). However, it is recommended that calcium gluconate be kept in stock in those labs where HF is being used. **See the following Honeywell document for medical emergency response information**:

http://www.honeywell.com/sites/servlet/com.merx.npoint.servlets.DocumentServlet?docid=DC63633A9-2871-90C6-BD48-1EE9B2F67D38

d. Exposure to HF must be immediately treated. HF exposure can result in serious injury, disfigurement, and/or death. HF is highly corrosive, as well as toxic and can continue to degrade tissue long after the initial exposure. Untreated exposure results in wounds that are difficult and very slow to heal and may result in deep-seated health problems. HF need only contact 2% of the body area to be fatal. Burns from strong HF (50-70% HF) are felt immediately; weaker solutions (25%) may take several minutes to be noticed; solutions of 1-20% may not be felt for several hours. Personnel assisting the injured should also ensure they are protected by personal protective equipment.

4. Mercury.

a. Containers of mercury must be closed when not in use.

b. All work involving mercury should be performed over trays or pans with turned-up edges to confine any spillage.

c. Mercury spills must be cleaned up immediately. The preferred method is to suction up the elemental mercury. A special vacuum cleaner for mercury spills is available from the Division of Public Safety. Please call for clean up of mercury spills. Ordinary vacuum cleaners must not be used because droplets will then be dispersed more finely throughout the laboratory.

d. Mercury monitoring can be accomplished by the Division of Public Safety.

e. Adequate ventilation must be provided. Vapors should not be re-circulated or exhausted to other laboratories or building areas.

B. Working with Radioactive Materials.

1. Complete information concerning the procurement, storage, use, handling, and disposal of any radioactive material can be found in the Radiation Safety Manual, which is available from the Division of Public Safety.

2. Personnel working with radioactive materials should maintain strict adherence to the general safety precaution as outlined above. In addition, the following steps must be taken:

a. Authorization for use of radioisotopes must be obtained by the faculty member

responsible for the research (Instructor or higher). Application is made to the Radiation Safety Committee. Authorization will cover only the radioisotopes, quantities, procedures, and areas requested.

b. All radioisotopes must be shipped to the Division of Public Safety, 108 Edwards Hall. Department personnel will deliver all shipments to the authorized laboratories.

c. Disposal of radioisotopes must be made through the Division of Public Safety. Radioactive waste is regularly picked-up weekly from the laboratories. Please call to be put on the pick-up list. Radioactive waste labels are available free from the Division. For pick-up, contact us through one of the following:

Phone: 2-5856
E-mail: safety@ksu.edu
Web: http://www.k-state.edu/safety

d. All laboratory personnel working with radioisotopes must be properly trained on an annual basis in the use of radioisotopes. Training is available on your department's local network. If not, contact the Radiation Safety Officer.

e. If a radiation meter is kept in the laboratory, it must be registered and calibrated annually. This process is provided by the Division of Public Safety.

C. Working with Lasers.

1. **L**ight **A**mplification by **S**timulated **E**mission of **R**adiation (laser) is a non-ionizing form of radiation. The laser is an intense, highly directional beam of light that can be directed, reflected, or focused on an object. The object will partially absorb the light, raising the temperature of the surface and/or interior of the object, and causing changes in the object. When the wavelength of the laser is in the ultraviolet (UV) region, then photochemical effects can occur in the object.

2. All Class II, IIIR (formally IIIA), IIIB, and IV laser systems used on campus must be registered with the Division of Public Safety.

3. Destruction of tissue can occur to the eye and skin. In the far-UV and far-infrared (IR) regions of the optical spectrum, the cornea will absorb the laser energy and be damaged. At certain wavelengths in the near-UV region and near-IR, the lens may be damaged. The greatest hazard is between 400 nm and 1400 nm where damage to the retina can occur. Light entering the eye from a collimated beam in the retinal hazard region is concentrated by a factor of 100,000 times when it strikes the retina. If the eye is not focused at a distance or if the laser light has been reflected off diffuse surfaces, this hazard is greatly diminished. Fires or vaporization of hazardous materials may result from laser beam interactions. Electrical shock is also a hazard in dealing with lasers and their power supplies.

4. Classification of lasers and laser hazards. Lasers are classified as continuous wave (CW) or repetitively pulsed (scanned). Energy can be emitted in the ultraviolet wavelengths (<400 nm), visible wavelengths (400-700 nm), or infrared wavelengths (>700 nm). There are four hazard classes of lasers or laser systems. See ANSI Z136.1 (2007) for complete classification definitions and Accessible Emission Limits (AEL's).

a. A Class I laser or laser system cannot emit levels of optical radiation above the

exposure limits for the eye under any exposure conditions inherent in the design of the laser product. There may be a more hazardous laser embedded in the enclosure of a Class I product, but no harmful radiation can escape from the enclosure. Class I lasers or laser systems are relatively safe, as long as the system is not modified.

b. A Class II laser or laser system emits a visible laser beam which, by its very bright nature, will be too dazzling to stare into for extended periods. Momentary viewing is not considered hazardous. The upper radiant power limit on this type of device is 1 milliwatt (mW) which corresponds to the total beam power entering the eye for a momentary exposure of 0.25 seconds. Class II lasers or laser systems require no special safety measures, *except that you must not stare into the beam*.

c. A Class III laser or laser system can emit any wavelength, but cannot produce a diffuse or scattered reflection hazard unless focused or viewed for extended periods at close range. It is also not considered a fire or serious skin hazard. Any CW laser that is not a Class I or II is considered a Class III device if its output power is ≤0.5 W. Since the output beam of such a laser is definitely hazardous for intrabeam viewing, control measures center on eliminating this possibility. Class III lasers or laser systems can produce a hazard if viewed directly. Safety training must be provided by the laboratory when using these lasers. In addition, the laser should be operated within a well marked and controlled area.

d. A Class IV laser or laser system is any that exceeds the AEL of a Class III device. These lasers may be either a fire or skin hazard or a diffuse reflection hazard. Class IV lasers or laser systems present a hazard not only from direct viewing of the beam or specular reflections, but also from the diffuse reflections. These lasers demand the use of eye protection, facility interlocks, and special safeguards.

5. The precautions in section 6 below apply to indoor laboratory use of lasers. Special precautions are required for outdoor laser use or laser use for entertainment purposes. Guidance is available from the Division of Public Safety.

6. General safety steps for all laser use.

a. NEVER stare into a laser beam.

(1) No safety glasses or goggles should be relied upon to view the direct laser beam. If laser safety goggles are needed for incident light, they should be suitable for the specific energy and wavelength of the laser beam in use.

(2) The laser room should be well lighted to keep the pupils of eyes as constricted as possible.

(3) Shield the laser so that beams cannot be seen at all but will be indirectly viewed, if necessary, by using an image converter either by inadvertence or through direct or diffuse reflection or scattering. For example, do not aim by sighting along the beam or confine reflections by using "barrier curtains."

(4) When high-power pulsed lasers are energized, a countdown system, with eyes closed during firing, should be used.

b. Be thoroughly familiar with the laser or laser system in use.

(1) Basic laser safety training must be provided to users by the department.

(2) Strictly follow a written standard operating procedure.

(3) Operators shall not permit specularly reflective materials to be placed in the beam path when not needed for the intended use.

(4) Each laser product, regardless of its class, must have a protective housing, which prevents access during normal operation.

(5) Each laser product or installation, regardless of its class, must be provided with a safety interlock for each portion of the protective housing which is designed to be removed or displaced during normal operation or maintenance.

(6) Equip laser capacitors with bleeder resistors to reduce the possibility of shock hazard.

c. Limit access to the area where the laser device is used.

(1) Each laser above Class I must provide visual or audible indication immediately before and during the emission of accessible laser radiation in excess of limits for Class I.

(2) Each controlled area must be posted with suitable warning signs.

(3) Use photocells that cut off the beam whenever the laser user intrudes into hazardous space, rather than just warning signs.

(4) Use a two-door laser room entry control, i.e., the first door opens into a vestibule. Occupancy of the vestibule is announced by a signal in the laser room. Exit from the vestibule through the second doorway into the laser room is possible only when all lasers in the room are off.

(5) Each laser product shall be secured against unauthorized operation.

(6) Each laser beam shall be terminated by material that does not allow laser radiation above the Maximum Permissible Exposure (MPE) limit in uncontrolled areas.

d. Be alert to medical problems.

(1) Personnel who are routinely exposed to potentially hazardous laser radiation should have an annual eye examination.

(2) Any afterimage, resulting from either a direct laser beam, or a reflection, should be reported to an ophthalmologist, preferably a specialist in retinal burns.

(3) When there is a reasonable probability that the MPE limit for the skin will be exceeded, the use of protective gloves, clothing, and shields is required.

(4) Users should strictly adhere to threshold limit values.

7. Associated hazards. Most incidents involving the use or operation of lasers are not due to the laser beam itself but to the associated hazards:

a. compressed gas cylinders;

b. carcinogens, such as dyes used for dye laser systems;

c. noise produced in some pulsed lasers, such as CO_2 lasers, can cause tissue shredding;

d. production of ionizing radiation;

e. Laser-Generated Air Contaminants (LGAC). When certain Class IIIB and Class IV laser beams interact with matter, air contaminants are formed. When the target irradiance reaches a given threshold, approximately 10^7 W/cm^2, target materials including plastics, composites, metals, and tissues, may liberate toxic or noxious airborne contaminants. LGAC include:

(1) polycyclic aromatic hydrocarbons from mode burns on poly-(methyl methacrylate) type polymers;

(2) hydrogen cyanide and benzene from cutting of aromatic polyamide fibers;

(3) fused silica from cutting quartz;

(4) mutagenic agents from laser surgery;

(5) heavy metals from etching;

(6) benzene from cutting polyvinyl chloride; as well as

(7) cyanide, formaldehyde, synthetic fibers, and natural fibers associated with other processes.

f. shards from shattering laser targets (therefore they must be enclosed);

g. shards from disintegrating bulbs, high-pressure arc lamps, or filament lamps;

h. ultraviolet radiation emitted from laser-discharge tubes; and

i. photosensitization. Some individuals are naturally photosensitive or are taking medication that induces photosensitivity.

D. Working with Ultraviolet Radiation.

1. Threshold Limit Values for UV sources subtending an angle less than 80°.

a. Total incidence on the unprotected eye in 315 to 400 nm.

(1) A radiant exposure of 1.0 J/cm^2 for periods lasting less than 1,000 seconds or

(2) an irradiance of 1.0 mW/cm^2 for periods lasting 1,000 seconds or more.

(3) Sources that subtend a greater angle need to be measured only over an angle of 80′.

b. Total incidence on the unprotected skin or eye at 180 to 400 nm should not exceed the following:

Wavelength (nm)	TLV (mJ/cm^2)
180	250
200	100
220	25

250	7
270	3
300	10
320	2,900
350	15,000
400	100,000

For assistance, contact the Division of Public Safety.

c. These limits should be used with caution when evaluating exposure to sunlight and do not apply to UV lasers, to the protection of photosensitive individuals nor aphakes, (persons who have had the lens of the eye removed in cataract surgery).

2. While ordinary spectacles will offer adequate eye protection in many cases, tinted safety glasses or goggles with solid sidepieces are recommended.

3. Skin protection can be accomplished by ordinary clothing.

4. Do not touch UV source envelopes. Skin oils can burn onto the glass and cause overheating of the glass envelope which may lead to implosion.

5. UV sources should be shielded to protect against flying glass shards.

E. Working with Microwaves.

1. Microwave equipment must be properly shielded. Any microwave equipment that is damaged, modified, or repaired must be tested for microwave leakage.

2. Confined heating of thermolabile materials may produce an explosion or fire, or generate gases which will be released to the laboratory.

3. Care should be taken when removing heated objects from a microwave oven, especially closed containers. Use of heat resistant gloves is recommended.

4. Hazards of microwaves.

a. Most commercial microwave ovens operate at a wavelength of ~12 cm (2450 MHz).

b. Irreversible damage to living tissue occurs at wavelengths ≥8 cm.

c. At a wavelength of ~10 cm, cataracts of the eye can occur with little or no sensation of heat at the time of irradiation.

d. Wavelengths ≥25 cm can cause an uncontrollable rise in body temperature.

e. Microwave radiation interferes with the operation of some pacemakers.

f. Microwave radiation will heat metal surgical implants which can become hot enough to destroy surrounding tissue.

F. Working with Chemical Carcinogens.

1. Information concerning the procurement, storage, use, handling, and disposal of any

chemical carcinogen can be obtained from the Division of Public Safety manual "Safety with Carcinogens in Research and Teaching."

2. Appendix B contains a list of actual or potential chemical carcinogens.

3. Personnel working with chemical carcinogens should maintain strict adherence to the general laboratory safety precautions.

G. Working with Biohazardous Materials.

1. Laboratory practice and technique.

a. All research and teaching using infectious agents (including Select Agents) must be reviewed by the Institutional Biosafety Committee (IBC) prior to project initiation.

b. Maintain strict adherence to standard microbiological practices and techniques.

c. Personnel working with infectious agents or materials must be aware of potential hazards and must be trained in the practices and techniques necessary for handling such material.

d. All Kansas State University employees who work with live vertebrates or infectious agents (Biosafety Level 2 or greater) must participate in the Occupational Health Biosafety Program (see Section X). Employees who feel that they may be at an increased risk of acquiring infection, or for whom infection might be unusually hazardous, must immediately advise their supervisor. To protect their own health, employees should seek the advice of their own physician.

e. The laboratory administrator must maintain a standard operating procedures manual which identifies the hazards and safe procedures designed to minimize or eliminate risks.

f. General safety practices include:

(1) Access to areas where infectious agents harmful to persons are used shall be limited to authorized personnel and appropriate warning signs will be posted.

(2) Personnel must wash their hands after they handle viable materials and animals, after removing gloves and before leaving the laboratory.

(3) Eating, drinking, smoking and applying cosmetics are not permitted in biological laboratories.

(4) Eye protection must be worn while working with pathogenic organisms. Personnel who wear contact lenses in laboratories should also wear goggles or a face shield.

(5) Laboratory coats, gowns or uniforms must be worn to prevent contamination or soiling of street clothes. Laboratory clothes must not be taken home to launder.

(6) Gloves must be worn if the skin on the hands is broken or if a rash exists.

(7) Food may not be stored in cabinets or refrigerators used for biological materials.

(8) Mouth pipetting is prohibited.

(9) All procedures must be performed carefully to minimize the creation of splashes or aerosols.

(10) Work surfaces must be decontaminated at least once a day and after any spill of viable material.

(11) All contaminated liquid or solid wastes or potentially infectious biological material will be autoclaved or properly disinfected prior to disposal. Materials must be transported in durable, leak-proof containers and closed for transport from the laboratory.

(12) An insect and rodent control program must be in effect.

2. Primary containment.

a. Must be provided to protect personnel and the laboratory environment from exposure to infectious agents.

b. Proper safety equipment must be provided for primary barriers. Safety equipment includes biohazard cabinets and other enclosed containers.

(1) The biological safety cabinet is the principal device used to provide containment of infectious aerosols. See VII.F for more information on biological safety cabinets.

(2) A safety centrifuge cup is an example of an enclosed container.

c. Personal protective equipment (PPE) such as gloves, coats, gowns, shoe covers, boots, respirators, face shields and safety glasses must be provided by your department. Use of PPE is required when working in certain animal studies, animal necropsy, pathogenic organisms, production activities or activities relating to maintenance, service or support of the laboratory facility.

d. Frequently, PPE must be worn while working in biological safety cabinets or other devices.

e. The use of vaccines may provide an increased level of personal protection.

3. Secondary barriers.

a. Secondary containment provides protection of the environment external to the laboratory from exposure to infectious materials.

b. Facility design protects personnel working inside and outside of the laboratory from infectious agents which may be accidentally released. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents.

4. Classification of Biosafety Levels. For more information, consult the most current edition of "Biosafety in Microbiological and Biomedical Laboratories." See Appendix D for biosafety levels appropriate for specific agents. Each level includes the requirements of all lower levels.

a. Biosafety Level 1 (BSL1). Appropriate for undergraduate training and teaching laboratories and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberiiv* and infectious canine hepatitis

virus are representative of those organisms. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains. BSL1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

b. Biosafety Level 2 (BSL2).

(1) Appropriate for clinical, diagnostic, teaching, and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Using good techniques, work with these agents can be safely conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatis B virus, the salmonellae and Toxoplasma spp. are representative of microorganisms assigned to this containment level. BSL2 is appropriate when work is done with any human-derived blood, body fluids, tissues or with human cell lines where the presence of an infectious agent may be unknown. Laboratory personnel working with human derived materials must be familiar with the Bloodborne Pathogen Program.

(2) Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Extreme precaution with contaminated sharps must be emphasized. Even though organisms routinely manipulated at BSL2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a biological safety cabinet or safety centrifuge cups. Other primary barriers must be used as appropriate, such as splash shields, face protection, gowns, and gloves.

(3) Secondary barriers such as hand washing and waste decontamination facilities must be available to reduce potential environmental contamination.

c. Biosafety Level 3 (BSL3).

(1) Appropriate for clinical, diagnostic, teaching, research or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious or potentially lethal infection. Examples of such agents include *Mycobacterium tuberculosis*, St. Louis encephalitis virus and *Coxiella burnetii.* Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

(2) Emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. Requires use of a biological safety cabinet or other enclosed equipment such as a gas tight aerosol generation chamber.

(3) Secondary barriers include controlled access to the laboratory and specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

d. Biosafety Level 4 (BSL4).

(1) ***Currently Kansas State University has no BSL4 facilities nor is it permitted to have any BSL4 facilities.***

(2) Practices, safety equipment, and facilities are applicable to work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL4 agents should also be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL4.

(3) BSL4 hazards include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment. Laboratory workers must be completely isolated from aerosolized infectious materials by working in a Class III biological safety cabinet or a full-body, air supplied positive-pressure personnel suit.

(4) BSL4 facilities are generally in a separate building or completely isolated zone with complex, specialized ventilation and waste management systems to prevent release of viable agents to the environment.

5. Human biological fluids or tissues should be treated as biohazardous agents to prevent the transmission of various human diseases such as Aquired Immune Deficiency Syndrome (AIDS), ARC, and Hepatitis B to laboratory personnel. All faculty, staff, and students working with human blood or human biological fluids contaminated with blood must be familiar with the Bloodborne Pathogen Program.

6. Restricted Animal Pathogens.

a. Non-indigenous pathogens of domestic livestock and poultry may require special laboratory design, operation, and containment features not generally addressed in the Centers for Disease Control and Prevention (CDC)-National Institute of Health (NIH) document “Biosafety in Microbiological and Biomedical Laboratories”. The importation, possession, or use of the following agents is prohibited or restricted by law or by U.S. Department of Agriculture (USDA) regulations or administrative policies:

African horse sickness virus;
African Swine fever virus;
Akabane virus;
Avian influenza virus;
Besnoitia besnoiti;

Bluetongue virus;
Borna disease virus;
Bovine spongiform encephalopathy;
Bovine infectious petechial fever agent;
Brucella abortus;
Brucellosis melitensis;
Burkholderia mallei (Pseudomonas mallei Glanders);
Camelpox virus;
Classical swine fever;
Cochliomyia hominivorax (screwworm);
Cowdria ruminantium (heartwater);
Creutzfeldt-Jacob Disease variant, Bovine spongiform encephalopathy;
Encephalopathy;
Ephermeral fever virus;
Foot and mouth disease virus;
Histoplasma (Zymonema farciminosum);
Louping ill virus;
Lumpy skin disease virus;
Mycobacterium bovis;
Mycoplasma agalactiae;
Mycoplasma mycoides (mycoides);
Nairobi sheep disease virus (Ganjam virus);
Newcastle disease virus (velogenic strains);
Peste des petits ruminants (pest of small ruminants) virus;
Rift Valley fever virus;
Riderspest virus;
Sheep and goat pox;
Swine vesicular disease virus;
Teschen disease virus;
Theileria annulata;
Theileria lawrencei;
Theileria bovis;
Theileria hirci;
Trypanosoma brucei;
Trypanosoma congolense;
Trypanosoma equiperdum (dourine);
Trypanosoma evansi;
Trypanosoma vivax;
Venezuelan equine encephalomyelitis virus;
Vesicular exanthema virus;
Vesicular stomatitis virus;
Viral hemorrhagic disease of rabbits; and
Wesselsbron disease virus.

b. The importation, possession, use, or interstate shipment of animal pathogens other than those listed above may also be subject to regulations of the USDA. Importation and shipment of all animal pathogens requires an import permit from USDA.

H. Working with Select Agents.

Specific rules are in place for the use of Select Agents at the University. Select Agents are those microorganisms and biological toxins (viruses, bacteria, rickettsiae, fungi, or toxins) that are considered capable of causing substantial harm to human health and are a threat to the United States. The list of Select Agents is found in Appendix E.

1. Each Principal Investigator, laboratory employee, room, and Select Agent used must be registered in the Select Agent Program. This can be done by contacting the Responsible Official at the Biosecurity Research Institute (BRI). The Responsible Official for Kansas State University is the Biosafety Officer at the BRI.

2. Laboratories that transfer or receive Select Agents must be registered with the Select Agent Program prior to transferring or obtaining Select Agents.

3. There are some exemptions for certain diagnostic or biomedical uses of these agents, but please check with the Responsible Official first. The exemptions are:

a. toxins for medical use;

b. agents inactivated for use as vaccines;

c. vaccine strains of viral agents (Junin Virus strain candid #1, Rift Valley fever virus strain MP-12, Venezuelan Equine encephalitis strain TC-83, Yellow fever virus strain 17-D);

d. vaccine strains as described in the Code of Federal Regulations (CFR) 9 CFR 78.1;

e. toxin preparations with an LD_{50} >100 ng/kg body weight for vertebrates, for use in biomedical research; and

f. products subject to regulation under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA).

4. Tracking of intrafacility transfers is the responsibility of the registered laboratory and it is required that the facility maintains adequate records of the transfers. The regulation is very clear that these agents may only be used or stored in a registered space and the Principal Investigator must notify the Responsible Official. Records must be maintained concerning every activity involved in working with, as well as the transferring and receiving of, Select Agents.

5. Proper security measures and safety practices must be followed. Security measures are outlined in the Kansas State University Select Agents and Toxins Security Plan available from the University Research Compliance Office or the Division of Public Safety.

6. This program is routinely inspected by the CDC and the USDA.

I. Working with Recombinant DNA Molecules.

1. Recombinant DNA research within the U.S. or its territories conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health (NIH) must comply with the most recent guidelines as published in the Federal Register. The guidelines specify practices for constructing and handling recombinant DNA molecules and organisms and viruses containing

recombinant DNA molecules.

2. The IBC has been established at Kansas State University in accordance with the NIH Guidelines to oversee recombinant DNA research. All research with recombinant DNA molecules must be registered with the IBC, even if those studies are exempt from the above guidelines. Information can be obtained from the University Research Compliance Office or the Division of Public Safety concerning the committee.

J. Working with Compressed Gas Cylinders.

1. Only those cylinders in immediate use shall be located in a laboratory. Replacement cylinders and empty cylinders shall be stored in a designated area, preferably outside the building. Do not store cylinders in hallways.

2. All compressed gas cylinders must be secured with a chain, clamp, or strap at all times when in use, storage, or transport.

3. Each tank must be properly and permanently identified when received. Never accept a cylinder on which the name of the contents is illegible. Do not rely on color codes for tank identification.

4. Do not attempt to modify or change cylinder valves or regulators.

5. Always use recommended handling procedures for compressed gas cylinders even though they may seem empty.

6. Empty or excess cylinders.

a. Return empty cylinders to the manufacturer, if possible.

b. Use all of the contents of the cylinder.

c. Cylinders that cannot be returned and are empty or are no longer wanted are handled through the hazardous waste program (see section IX). Contact the Division of Public Safety.

K. Working with Cryogenic Liquids.

1. Storage and handling of cryogenic liquids is similar to compressed gases.

2. Store in a well ventilated area to prevent buildup of gases or displacement of air. Do not store cryogenic liquids in hallways.

3. Avoid contact with moisture to prevent ice plugging of relief devices.

4. Keep all sources of ignition away.

5. Always wear eye protection, preferably a face shield and goggles.

6. Do not wear gloves which can be frozen to the skin - use a potholder.

7. Provide good ventilation in the use area.

8. Provide venting for Dewar flasks used in experiments.

9. Use care in transporting fragile cryogenic containers. Use a hand truck for transport.

10. Select work materials carefully. Cryogenic temperatures may alter the physical characteristics of many materials.

11. Use only approved storage vessels with pressure relief valves.

IX. DISPOSAL OF CHEMICAL WASTE

A. Introduction.

1. The safe use and disposal of chemicals is required of everyone. Chemicals are used in every department on the Kansas State University campus. It is the legal responsibility of each faculty, staff, and student at the University to deal with chemicals properly.

2. The EPA enforces various laws such as RCRA, the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or Superfund), the Superfund Amendment Reauthorization Act (SARA) and TSCA which are meant to protect the environment. The EPA encourages chemical waste minimization in every industry. The Kansas State University administration agrees with the EPA for the need to reduce the quantities of hazardous waste that are generated. With this in mind, the Hazardous Waste Minimization Policy was written and is enforced (see section IX.H. below).

3. Penalties. The department or college that allows the improper storage or disposal of chemicals or chemical products will be held liable for any fees or penalties imposed by the EPA or the Kansas Department of Health & Environment (KDHE). Any costs for waste disposal that may have been prevented by proper recycling may be imposed on the offending department or college by the Division of Public Safety.

4. Individuals (faculty, staff or students) who have knowledge of improper disposal of chemicals or chemical products must make the University administration aware of the situation. This can be accomplished by advising the Division of Public Safety, any member of the Campus Environmental Health & Safety Committee, or the K-State Maintenance and Service Employee Safety Committee. The University guarantees that no reprisal will be held against the individual.

B. Hazardous waste is defined under RCRA and the Hazardous & Solid Waste Amendments (HSWA) as any liquid, solid or gas that has no commercial value (solid waste) and has a hazardous characteristic component. These characteristics are:

1. Ignitability (flash point less than 140°F);

2. Corrosivity (pH less than or equal to 2 or greater than or equal to 12.5);

3. Reactivity (reacts with air or water to produce an explosive, flammable or toxic product); or

4. Toxicity (contains specific pesticides, heavy metals or organic solvents).

C. In addition to the above characteristics, chemicals that are hazardous waste also identified in the F-list, K-list, P-list and U-list. Chemicals must be handled correctly when they are to be discarded. Kansas State University is regulated as a generator of hazardous waste (EPA Generator status) and must comply with the laws governed by KDHE and the EPA. Chemicals may not be haphazardly discarded down the sink drain, poured onto the ground, discharged to the atmosphere, or buried at an unpermitted site.

D. These guidelines will enable the user to directly process and discard most materials appropriately or minimize hazardous waste. A pick up service is provided by the Division of Public Safety for proper disposal. For additional information or help in disposing of

chemicals call the Division of Public Safety, 2-5856.

1. All chemicals must be identified with the name (not symbols, formulae, or abbreviations). This includes hazardous waste.

2. All waste containers must be marked with the words "Hazardous Waste."

3. All containers of hazardous waste must be kept closed.

4. Hazardous waste must be kept in the room where the waste is generated.

5. Full containers of hazardous waste must be dated when full and removed from the laboratory within three (3) days of the date.

E. Waste awareness training is provided by the University. One-hour classes are held monthly in the basement training room in Edwards Hall. Departments may request a seminar at their location and training is available on the Division of Public Safety web page (http://www.k-state.edu/safety). All employees are encouraged to attend this training at least once.

F. Responsibilities.

1. The person responsible for the generation of the waste or the person in charge of the materials should handle the chemical waste.

2. Use and recycle materials in their intended fashion to limit disposal problems.

3. Each laboratory should be equipped with a fume hood and the means to carry out simple neutralization reactions.

4. Empty containers should be rinsed three times and then properly discarded to a trash dumpster. Deface the label on empty containers so there is no question about hazards. Such empty containers are not normally considered hazardous waste (see IX.G.5 below).

G. Empty containers. Containers or container liners that held hazardous materials are not usually regulated as hazardous waste if they are empty. A container is considered empty if:

1. All wastes have been removed that can be removed by pouring, pumping, and aspirating, and

2. no more than one inch of residue remains in the container, or

3. no more than 3.0% by weight of the contents remain inside the container (≤110 gallons), or

4. no more than 0.3% by weight of the contents remain inside the container (>110 gallons);

5. Any container that held P-listed materials or wastes must be triple rinsed before they are considered empty. The rinsate must be collected and handled as hazardous waste. Non-rinsed P-listed material containers may be turned in for hazardous waste disposal. See Appendix F for P-listed chemicals.

H. Hazardous Waste Minimization.

1. Laboratories are accountable for their hazardous waste from the time it is generated until it no longer exists. The one method to ensure proper disposal is to reduce or

minimize the waste produced. The EPA requires waste minimization as a method of pollution prevention. The Kansas State University administration agrees with the EPA for the need to reduce the quantities of hazardous waste generated. Management and staff at all levels must be openly and actively committed to supporting sound waste management policies and practices.

2. The success of the Waste Minimization Plan depends ultimately on the participation and cooperation of all laboratory workers and students. The K-State community must do everything that is within its means to reduce hazardous waste on the campus. Each campus unit is urged to use any of the following techniques to reduce hazardous waste:

a. substitution (use a less hazardous chemical)

b. small quantity purchases

c. microscale experiments

d. redistribution (use of recycled chemicals)

e. waste segregation (keep waste streams separate, for example, separate organic from aqueous or halogenated from nonhalogenated).

f. Re-use waste as is or after purification. Certain purification processes such as distillation are permitted in the laboratories. The waste from these processes should be handled as hazardous waste. For assistance, contact the Division of Public Safety.

3. Acids and bases.

a. Laboratory acids or bases should not be haphazardly discarded. Most acid and base wastes can be neutralized to a pH 7±□2 and washed down the sink drain with copious amounts of water.

b. There are many alternatives today to the use of chromic acid cleaning baths. There are many suitable non-chromate containing substitutes available on the market that are safer and just as effective as chromic acid.

4. Batteries. No batteries may be discarded in the trash. Batteries will be recycled if possible by the Division of Public Safety. An effort will be made to return dead or weak batteries to the manufacturer or a battery recycler. If no means are found to recycle batteries, they may be discarded as hazardous waste by the Division of Public Safety.

5. Bottled gas. Gas cylinders should only be procured from dealers or manufacturers that will accept the return of empty cylinders (see section VIII. J above). This is especially important in the use of lecture size or smaller bottles. Aerosol cans are considered to be gas cylinders. Empty aerosol cans may be discarded in the trash. For assistance, call the Division of Public Safety.

6. Oil. Used oil from vehicles, machines, pumps, compressors, etc., must be recycled. Used oil may not be dumped on the ground for weed or dust control. Currently, there are several used oil-burning space heaters in use on campus. Other farm or maintenance units on campus are encouraged to install used oil-burning space heaters. To dispose of used oil, call the Division of Public Safety.

7. Paints. Waste petroleum-based paints or paints containing lead, silver, chromium or

other toxic heavy metals must be disposed of as hazardous waste. Use latex paints rather than petroleum solvent-based paints. Use alternatives to paints containing chromates. Do not buy and store large quantities of petroleum-based paints. Use up all paints; do not leave small quantities that will require hazardous waste disposal. Liquid latex paint may not be discarded in the trash, it must first be dried. The Division of Public Safety will also pick up and recycle latex paint.

8. Solvents. The use of organic solvents in the laboratory is very common. Laboratory personnel should consider the purchase and operation of solvent stills or high performance liquid chromatography (HPLC) solvent recyclers to reclaim used solvent.

9. Pesticides. Do not buy or request more pesticides than is necessary for the research. Use less toxic or less flammable pesticides if possible. Limit the amounts kept in storage to prevent disposal of out-dated pesticides. Arrange for the return of research pesticides to the manufacturer. Old or unwanted pesticides will be recycled by the Division of Public Safety.

10. Photographic waste. Silver waste from photographic development is considered hazardous waste and must not be discarded into the sink drain. For assistance, call the Division of Public Safety.

11. Mercury.

a. Do not discard elemental mercury or glassware contaminated with mercury in the trash. Do not sprinkle sulfur on mercury spills. Contact the Division of Public Safety to clean up mercury spills. Mercury and mercury-contaminated materials are recycled by the Division of Public Safety.

b. Departments are encouraged to replace mercury containing devices such as manometers, barometers, thermometers, etc., with non-mercury devices. The Division of Public Safety can help with your replacement program.

12. Laboratory chemicals. Each laboratory that uses chemicals must make an effort to reduce shelf stock wherever possible. A great deal of Kansas State's hazardous waste is from the clean out of vacated laboratories. This waste generation could have been minimized if greater care were spent in purchasing, stocking, and using chemicals.

a. Purchase only what is needed and maintain a current inventory of chemicals in stock. The Division of Public Safety expects laboratory managers to use inventories as a means to reduce waste. Buy only the quantities of chemicals needed without overstocking, i.e., buy one bottle rather than one case or 100 ml rather than 1 liter.

b. Use up old chemical stock before buying new stock.

c. Establish a centralized chemical storage area. This would facilitate the redistribution of surplus chemicals. Buy chemicals at the established chemical storerooms on campus such as the Biology Storeroom (Ackert Hall) or the Chemistry Storeroom (King Hall).

d. Promptly replace deteriorated labels and containers. No chemical container may be kept in storage without an identifying label.

e. The Division of Public Safety has instituted a university wide system for chemical

inventory. This system is available via the web for all university employees with research or teaching laboratory space. All laboratories are asked to use this system.

To enter into the system, see the EH&S Assistant icon on the Division of Public Safety webpage (http://www.k-state.edu/safety).

I. In-Lab Disposal.

1. Neutralization. Many laboratory chemicals can be neutralized or made nonhazardous in the laboratory and discarded into the sanitary sewer system. For instance, mineral acids may be neutralized with a base to a pH between 5 and 9. The resulting solution may then be washed down the sink drain with copious amounts of water. For additional information or help in neutralizing chemicals, call the Division of Public Safety. Several books have been published that suggest laboratory neutralization processes, some noted ones are:

a. Prudent Practices in the Laboratory;

b. Hazardous Laboratory Chemicals Disposal Guide;

c. Destruction of Hazardous Chemicals in the Laboratory; and

d. Waste Disposal in Academic Institutions

2. Evaporation. Do not dispose of chemicals by evaporation. *Evaporation of solvents or other chemicals as a means of disposal is not permitted under RCRA.*

3. Sanitary sewer system (the sink drain). Very few chemicals may be discarded into the sanitary sewer.

Only small quantities of non-flammable, low hazard, biodegradable and water-soluble materials may be disposed this way. Amounts of less than 100 g or 100 ml per day may be flushed down the drain with copious amounts of water.

4. Trash dumpster. Do not dispose of chemicals in the trash.

J. Hazardous Material Pick Up.

1. A pick up service is provided by the Division of Public Safety. Appropriate labels are available at no charge from the Division of Public Safety.

a. Waste material may be collected in empty compatible glass or plastic containers.

b. Waste collection containers must be marked with the words "***Hazardous Waste***."

c. Waste collection containers must be marked with the date when full.

d. Waste collection containers must be kept closed except when adding waste.

e. Halogenated waste must be kept separate from non-halogenated waste.

f. Organic waste should be kept separate from inorganic waste.

g. "Defuse" reactive waste in the laboratory when it is appropriate.

h. Keep waste materials from different processes separate, if possible.

i. Biological (Biohazardous) Materials. Pathogenic organisms or contaminated materials must be sterilized prior to disposing in the trash. Materials designated as

"bloodborne pathogen contaminated" must be disposed of as medical waste. This includes sharps (needles, syringes, scalpels, razors, Pasteur pipettes, etc.). Contact the Division of Public Safety for assistance.

j. Excess, off-spec, or out-dated unused laboratory reagents for pick-up do not need to be labeled "Hazardous Waste" as long as there is a chemical identification label on the container.

2. In preparation for hazardous material pick up:

a. collection containers for hazardous waste must be labeled with the words "Hazardous Waste," marked with the name of the chemical contained, dated when full, and be closed to prevent spillage'

b. non-compatible materials must be kept separated (see Appendix A);

c. box groups of containers so that they can be carried easily by hand;

d. label the box "PUBLIC SAFETY - WASTE"

3. Request hazardous waste pick up from the Division of Public Safety.

Phone: **2-5856**;
E-mail: **safety@ksu.edu**
Web: **http://www.k-state.edu/safety**

X. OCCUPATIONAL HEALTH AND SAFETY PROGRAM

The University Research Compliance Office (URCO) coordinates the Occupational Health and Safety Program for persons using live vertebrates in research, testing, and teaching. This program is mandated by The U.S. Public Health Service (PHS) and requires everyone (employees/students) engaged in animal care and use programs at KSU to enroll in the Occupational Health and Safety Program. This process is a risk based evaluation that is conducted by an Occupational Health Physician at Mercy West Hospital. All necessary forms are located at URCO web site - http://urco.ksu.edu. If you have any questions, please contact the URCO by email at: comply@ksu.edu or by phone at 2-3224.

A. Accident/Incident Reporting – After supervisor has been notified:

1. All personnel must report cuts, scratches, bites, or other job related injuries associated with animal care and use programs within 24 hours of the injury. The proper accident report must be filed with the employee's department, URCO and Division of Public Safety.

- PER-17, form is located at: http://www.k-state.edu/hr/forms/per17.pdf
- Memo for Record, form is located at: http://urco.ksu.edu.

2. All personnel who work with BSL-2 or greater organisms that have had potential exposures to those organisms in the laboratory must complete accident report with their department, URCO, Division of Public Safety and see a physician at Mercy Regional Health Center if appropriate.

- PER-17, form is located at: http://www.k-state.edu/hr/forms/per17.pdf
- Memo for Record, form is located at: http://urco.ksu.edu.

B. Mandatory Training. There are mandatory training programs for occupational users of animals and biological agents. These programs are administered by the URCO and are located at http://urco.ksu.edu. Workers must be trained and proficient in the practices and techniques required for handling live vertebrates and biohazards safely. Live vertebrates present some unique problems that do not exist in the laboratories, such as: production of aerosols, biting, scratching, or kicking.

1. Animal Care and Use Training (IACUC) - http://urco.ksu.edu
2. Institutional Biosafety Training (IBC) - http://urco.ksu.edu
3. Bloodborne Pathogen Program - http://www.k-state.edu/policies/ppm/3720.html#bloodbrn

XI. BIBLIOGRAPHY

ACGIH, American Conference of Governmental Industrial Hygienists. TLVs, 2008 Threshold Limit Values and Biological Exposure Indices. ACGIH, Cincinnati, OH. 2008.

ACS, American Chemical Society. Safety in Academic Chemistry Laboratories. 1985.

ANSI, American National Standards Institute, Z136.1, Laser Accessible Emission Limits, 2007.

Armour, M.A., Hazardous Laboratory Chemicals Disposal Guide. CRC Press, Inc., Boca Raton, FL. 1991.

CDC/NIH, Center for Disease Control/National Institute of Health. 5th Edition Biosafety in Microbiological and Biomedical Laboratories. US Government Printing Office, Washington, DC. 2007.

Clayton, G. D., Clayton, F. E., ed. Patty's Industrial Hygiene and Toxicology. John Wiley & Sons, New York. 1978-85 (6 volumes).

Dornhoffer, M. K. Handling Chemical Carcinogens: A Safety Guide for the Laboratory Researcher. Chemsyn Science Laboratories, Lenexa, KS. 1986.

EPA, Environmental Protection Agency. Hazardous Waste Management Regulations, 40 CFR 260-271. US Government Printing Office, Washington DC. 2007.

International Technical Information Institute. Toxic and Hazardous Industrial Chemicals Safety Manual. Tokyo, Japan. 1979.

IUPAC-IPCS, International Union of Pure and Applied Chemistry - International Programme on Chemical Safety, Chemical Safety Matters. Cambridge University Press, Cambridge. 1992.

Kaufman, J. A., ed., Waste Disposal in Academic Institutions. Lewis Publishers, Chelsea, MI. 1990.

KDHE, Kansas Department of Health and Environment. Hazardous Waste Generator Handbook. 2007.

Lunn, G. and Sansone, E. B., Destruction of Hazardous Chemicals in the Laboratory. John Wiley & Sons., Inc., New York. 1990.

Manufacturing Chemists Association. Guide for Safety in the Chemical Laboratory. Van Nostrand Reinhold, New York. 1972.

Matheson, Guide to Safe Handling of Compressed Gases. Matheson Gas Products, Inc. 1983.

NACUBO, National Association of College and University Business Officers. Hazardous Waste Management at Educational Institutions. NACUBO, Washington, DC. 1987.

NRC, National Research Council. Prudent Practices in the Laboratory, Handling and Disposal of Chemicals. National Academy Press, Washington, DC. 1995.

NCI, National Cancer Institute. Biological Safety Manual for Research Involving Oncogenic Viruses, DHEW Publ. No. (NIH) 76-1165. US Government Printing Office, Washington, DC. 1976.

NFPA, National Fire Protection Association. Fire Protection for Laboratories Using Chemicals,

NFPA 45. NFPA, Boston, MA. 1996.

NFPA, National Fire Protection Association. Fire Protection Guide on Hazardous Materials, NFPA 704. NFPA, Quincy, MA. 2001.

NFPA, National Fire Protection Association. Hazardous Chemicals Data, NFPA 49. NFPA, Quincy, MA. 2001.

NFPA, National Fire Protection Association. Life Safety Code, NFPA 101. NFPA, Quincy, MA. 2009.

NIH, National Institute of Health. Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). US Government Printing Office, Washington, DC. 1996.

NIH, National Institutes of Health. NIH Guidelines for the Laboratory Use of Chemical Carcinogens, NIH Publ. No. 81-2385. US Government Printing Office, Washington, DC. 1981.

OSHA, Occupational Safety and Health Administration. General Safety Standards, 29 CFR 1910. US Department of Labor. 2008.

OSHA, Occupational Safety and Health Administration. Health and Safety Standards; Occupational Exposures to Toxic Substances in Laboratories; 29 CFR 1910.1450. Federal Register, July 24, 1986.

Pipitone, D. A., ed., Safe Storage of Laboratory Chemicals. John Wiley & Sons, Inc., New York. 1991.

Reinhardt, P. A., Leonard, K. L., and Ashbrook, P. C., Pollution Prevention and Waste Minimization in Laboratories. CRC Press, Inc., Boca Raton, FL. 1995.

Sax, N. I., ed. Dangerous Properties of Industrial Materials. Van Nostrand Reinhold, New York. 1995

Sontag, J. M., Page, N. P., Saffiotti, U. National Cancer Institute Guidelines for Carcinogen Bioassay in Small Rodents, NCI-CG-TR-1. DHEW publ. No. (NIH) 76-801. US Government Printing Office, Washington, DC. 1976.

Steere, N. V., ed. CRC Handbook of Laboratory Safety. Chemical Rubber Company, Cleveland, OH. 1967.

Windholz, M., Budavari, S., Stroumtsos, L., Fertig, M. N., ed. The Merck Index. Merck & Co., Rahway, NJ. 1986.

Young, J. A., Improving Safety in the Chemical Laboratory, A Practical Guide. John Wiley & Sons, Inc., New York. 1991.

APPENDIX A

CHEMICAL COMPATIBILITY

DO NOT CONTACT:	WITH:
Alkali Metals	Water, carbon dioxide, calcium, potassium, carbon tetrachloride, sodium, and other chlorinated hydrocarbons.
Acetic Acid	Chromic acid, nitric acid, hydroxyl-containing compounds, ethylene glycol, perchloric acid, peroxides, and permanganates.
Acetone	Concentrated sulfuric and nitric acid mixtures.
Acetylene	Copper (tubing), fluorine, chlorine, iodine, silver, mercury, and their salts.
Ammonium Nitrate	Acids, metal powders, flammable liquids, chlorates, nitrates, sulfur, and finely divided organics or combustibles.
Anhydrous Ammonia	Mercury, halogens, calcium hypochlorite, and hydrogen fluoride.
Aniline	Nitric acid, hydrogen peroxide.
Bromine	Ammonia, acetylene, butadiene, butane, hydrogen, sodium carbide, turpentine, and finely divided metals.
Chlorates	Ammonium salts, acids, metal powders, sulfur, finely divided organics or combustibles, and carbon.
Chromic Acid	Acetic acid, naphthalene, camphor, alcohol, glycerine, turpentine, and other flammable liquids.
Chlorine	Ammonia, acetylene, butadiene, benzene and other petroleum fractions, hydrogen, sodium carbide, turpentine, and finely divided powdered metals.
Cyanides	Acids.
Hydrogen Sulfide	Nitric acid, oxidizing gases.
Hydrocarbons	Fluorine, chlorine, bromine, chromic acid, and sodium peroxide.
Iodine	Ammonia, acetylene, butadiene, benzene and other petroleum fractions, hydrogen, sodium carbide, turpentine, and finely divided powdered metals.
Mercury	Acetylene, fulminic acid, and hydrogen.
Nitric Acid	Acetic, chromic and hydrocyanic acids, aniline, carbon, hydrogen sulfide, flammable liquids or gases, and substances which are readily nitrated.
Oxygen	Oils, grease, hydrogen, flammable liquids, solids or gases.

Oxalic Acid	Silver and mercury.
Perchloric Acid	Acetic anhydride, bismuth and it's alloys, alcohol, paper, wood, and other organic materials.
Phosphorous Pentoxide	Water.
Potassium Permanganate	Glycerine, ethylene glycol, benzaldehyde, and sulfuric acid.
Sodium Peroxide	Any oxidizable substances, e.g., methanol, glacial acetic acid, acetic anhydride, benzaldehyde, carbon disulfide, glycerine, ethylene glycol, ethylacetate, and furfural.
Sulfuric Acid	Chlorates, perchlorates, permanganates, and water.

APPENDIX B

CHEMICAL CARCINOGEN LIST

Examples of chemical carcinogens, potential carcinogens and tumor promoters as listed in the Safety with Chemical Carcinogens in Research and Teaching Manual.

N-acetoxy-2-acetamidofluorene
N-acetoxy-2-acetamidostilbene
N-acetoxy-4-acetamidobiphenyl
N-acetoxy-N-acetamidophenanthrene
2-acetylaminofluorene
acrylonitrile
actinomycin D
adriamycin
aflatoxins
aldrin
alkanes (certain long chain ones)
allyl methylsulfonate
alpha,alpha-dichloromethyl methyl ether
anthralin
2-aminoanthraquinone
4-aminobiphenyl
1-amino-2-methylanthraquinone
amitrole
o-anisidine
o-anisidine hydrochloride
aramite
arsenic and certain arsenic compounds
asbestos
auramine (technical grade)
azathioprine
benz[a]anthracene
benzene
benzidine and benzidine based dyes
benzo[c]chrysene
benzo[b]fluoranthene
benzo[j]fluoranthene
benzo[k]fluoranthene
benzo[g,h,I]perylene
benzo[O]phenanthrene
benzo[a]pyrene
benzotrichloride
benzoyl peroxide
beryllium and certain beryllium compounds
N,N-bis(2-chloroethyl)-2-naphthylamine
bischloroethyl nitrosourea (BCNU)
bischloromethyl ether (BCME)
7-bromomethylbenz[a]anthracene
3-bromopropionic acid
1,3-butadiene
1,4-butanediol dimethane sulfonate
N-butyl-N-nitrosourethane
cadmium and certain cadmium compounds
carbon black
carbon tetrachloride
carrageenan (degraded)
chlorambucil
chloramphenicol
chloroacetone
1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)
chloroethylene oxide
chloroform
chloromethyl methyl ether (technical grade)
chlorophenols
chloroprene
chromium and certain chromium compounds
chrysarobin
chrysene
cisplatin
citrus oils
coal gasification
coal liquefaction
coal-tar products
coal-tar pitch volatiles
coke oven emissions
coke production
conjugated estrogens
p-cresidine
croton oil
cupferron
cycasin
cyclophosphamide
dacarbazine
DDT
2,4-diaminoanisole and its salts
2,4-diaminotoluene
dibenz[a,h]acridine
dibenz[a,j]acridine
dibenz[a,h]anthracene
7H-dibenzo[c,g]carbazole
dibenzo[a,j]anthracenen
dibenzo[a,c]naphthacene
dibenzo[a,e]pyrene
dibenzo[a,h]pyrene
dibenzo[a,i]pyrene
dibenzo[a,l]pyrene
1,2-dibrom-3-chloropropane (DBCP)
3,3'-dichlorobenzidine and its salts
dieldrin
dienestrol
diepoxybutane
1,2,4,7-diepoxyhexane
1,2,4,5-diepoxypentane
di(2,3-epoxypropyl) ether (DGE)
di(2-ethylhexyl)phthalate

diethylstilbestrol (DES)
diethyl sulfate
dihydroteleocidin B
3,3'-dimethoxybenzidine
4-dimethylaminoazobenzene
dimethylcarbamoyl chloride
1,1'-dimethyl hydrazine
dimethyl sulfate
1,4-dioxane
direct black 38, technical
direct blue 6, technical
direct brown 95, technical
epichlorohydrin
1,2-epoxybutyronitrile
estradiol-17B
estrone
ethinylestradiol
ethylene dibromide (EDB)
ethylene dichloride (EDC)
ethyleneimine
ethylene oxide
ethylene thiourea
ethyl methanesulfonate
1-ethyl-1-nitrosourea
euphorbia lattices (certain ones)
fatty acids and fatty acid methyl esters (certain ones)
1-fluoro-2,4-dinitrobenzene
formaldehyde
2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide
glycidaldehyde
gyromitrin
hexachlorobenzene
hexachlorobutadiene
hexachloroethane
hexamethyl phosphoramide
hydrazine
hydrazine sulfate
hydrazobenzene
hexachlorobutadiene
hexachloroethane
N-hydroxy-2-aminoaphthalene
beta-hydroxy-1-ethylaziridine
ICR 170
indeno[1,2,3-cd]pyrene
iodoacetic acid
iron dextran complex
kepone (chlordecone)
lead acetate
lead phosphate
lindane and other hexachlorocyclohexane isomers
malonaldehyde
melphalan
mestranol
7-methylbenz[a]anthracene
methyl bromide
methyl chloride
3-methylcholanthrene
5-methylchrysene
11-methylcyclopenta[a]phenanthren-17-one
4,4'-methylenebis(2-chloroaniline) (MOCA)
4,4'-methylenebis(n,n-dimethyl) benzenamine
4,4'-methylene dianiline
methyl hydrazine
methyl iodide
methyl methanesulfonate
N-methyl-N'-nitro-N-nitrosoguanidine
4-O-methyl tetradecanoylphorbol-13-acetate
N-(4-methoxy)benzoyloxy piperadine
N-methyl-N'-nitro-N-nitrosoguanidine
metronidazole
mezerein
michler's ketone
mineral oils
mirex
mitomycin C
mustard gas
alpha-naphthylamine
beta-naphthylamine
nickel carbonyl
nickel and certain nickel compounds
nickel sulfide roasting, fume and dust
nitrilotriacetic acid
5-nitro-o-anisidine
N-(4-nitro)benzoyloxypiperadine
4-nitrobiphenyl
nitrofen
nitrogen mustard
2-nitropropane
4-nitroquinoline-N-oxide
N-nitrosodimethylamine
N-nitrosodi-n-butylamine
N-nitrosodiethanolamine
N-nitrosodiethylamine
N-nitrosodimethylamine
p-nitrosodiphenylamine
N-nitrosodi-n-propylamine
N-nitroso-n-ethylurea
N-nitroso-n-methylurea
N-nitrosomethylvinylamine
N-nitrosomorpholine
N-nitrosonornicotine
N-nitrosopiperidine
N-nitrosopyrrolidine
N-nitrososarcosine
norethisterone
oxymetholone
pentachloronitrobenzene
phenactin
phenazopyridine
phenazopyridine hydrochloride
phenolic compounds (certain ones)
phenoxyacetic acid

herbicides
N-phenyl-beta-naphthylamine
phenylhydrazine
phenytoin
phorbol-12,13-dibenzoate
phorbol-12,13-didecanoate
polybrominated biphenyls (PBB)
polychlorinated biphenyls (PCB)
procarbazine
procarbazine hydrochloride
progesterone
propane sultone
beta-propiolactone
propyleneimine
propylthiouracil
reserpine
12-O-retinoylphorbol-13-acetate
saccharin
safrole
selenium sulfide
sodium lauryl sulfate
soots, tars, and mineral oils
sterigmatocystin
streptozotocin
sulfallate
teleocidin
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
1,1,2,2-tetrachloroethane
tetrachloroethylene
2,3,4,5-tetrachloronitrobenzene
2,3,4,6-tetrachloronitrobenzene
2,3,5,6-tetrachloronitrobenzene
12-O-tetradecanoylphorbol-13-acetate
thioactamide
thiourea
thorium dioxide
tobacco extracts and condensates
o-tolidine
o-toluidine
o-toluidine hydrochloride
p-toluidine
toxaphene
trenimone
treosulphan
1,1,3-trichloroethane
2,4,6-trichlorophenol
trichlroethylene
triethylenemelamine
tris(aziridinyl)-p-benzoquinone
tris(aziridinyl)phosphine sulfide
tris(2,3-dibromopropyl)phosphate
tryptophane P1
tryptophane P2
Tween 60
uracil mustard
urethane
vinyl bromide
vinyl carbamate
vinyl chloride
vinyl cyclohexene dioxide
vinyl fluoride
vinylidene chloride
vinylidene fluoride monomer

APPENDIX C

SPILL RESPONSE CART

The following hazardous material spill response cart was designed by C.W. Stinebaugh, Ford Motor Co. Spill carts can also be purchased from various laboratory and safety supply dealers.

Cart dimensions should be 36" W x 48" L x 48" H with principal components fabricated of fiberglass or fiberglass reinforced plastic (FRP). Doors or other lockable closures are suggested because many items stocked on the cart are of high value. An alternative to all-side enclosures is the use of a suitable cover for the entire cart or storing the cart in a secure room. If it is deemed necessary to carry supplies on top of the cart, a 1" perforated rail should be used as a perimeter retainer. The primary weight-bearing wheels and rear steering casters are commercially available items as follows:

Front Wheels (2) - 10" x 3" made of polyolefin material with rollers bearings.

Rear Wheels (2) - Stainless steel construction, double ball bearing swivel casters with parking brake.

The recommended contents of the cart and their purpose are listed below. All items are commercially available or readily prepared.

EMERGENCY RESPONSE KIT. This includes a red hard hat, 2 each polylaminated impervious coveralls (Tyvek), 2 each nitrile gloves, 2 each no vent goggles, half mask respirator for dusts and acid gases/organic vapors, and safety flashlight with batteries. An extra pair or two of coveralls, gloves, goggles, flashlight and batteries and respirator should be kept in storage. A small-bottle, self-contained breathing apparatus (SCBA) should also be considered.

CHEMICAL ABSORPTION MOP (1 EACH). Disposable, chemically resistant sponge that absorbs acid, caustic and oil spills. Has plastic handle and works like a normal squeeze-action mop, but is made entirely of non-sparking materials. Keep carton of 12 replacement sponges in reserve.

DISPOSAL BAGS (12 EACH). Polyethylene bags (17" x 30", 2 mil thick) with hazardous markings for the disposal of spill saturated absorbent. Keep some in reserve.

DRY, ANGULAR GRAIN CLAY-TYPE ABSORBENT (Cat Litter) (20 POUNDS). Use for traction in slippery areas and as an absorbent. Recommend 2 bags reserve supply.

SPILL PILLOWS (6 EACH, 1 LITER CAPACITY). Use for absorption/solidification of spilled materials. Solidification process neutralizes acids and reduces flammability, corrosivity, reactivity, and toxicity of liquids. Store six in reserve.

DIKING MATERIAL (1 EACH). Shredded polypropylene in 3" diameter, 12' long molded tubes packed in poly bags. Store two in reserve storage.

VAPOR BARRIER SORBENT BLANKETS (25 FEET). Blanket sheets are of highly absorbent polypropylene microfibers with polyethylene film on one side to help suppress and contain vapors of acid, base, solvent and oil spills. 12" x 150' roll. Keep balance on the roll in reserve.

SPILL SQUEEGEE (1 EACH). 18" polypropylene blade with wooden handle.

SODIUM BICARBONATE. Maintain an appropriate amount (determined by conditions to be encountered) on the cart as an acid neutralizer, with some held in reserve storage.

DRAIN STOPPER (1 EACH). 24" x 24" elastomer material floor or surface mounted drain cover. Flexible to fit drain lid contour and is a gravity seal that is chemically resistant and washable.

SPILL CONTROL EXTINGUISHER (1 EACH). For use on spills of flammable liquid. Spreads an aqueous foam over the spill, which provides a vapor seal to inhibit ignition. 2 gallon capacity. Hold package of liquid premix in reserve to service refill requirements.

SPARK-FREE SHOVEL (1 EACH). 38" overall length with 11" wide blade of solid polypropylene material, is extremely rugged yet lightweight and resists chemicals and corrosion.

DUST PAN AND BROOM (1 EACH). Broom has polypropylene bristles and dust pan is lobby-type scoop with handle. Both have chemically resistant polyethylene housing and wood handles.

BARRICADE TAPE (1 ROLL). 3" x 1000' roll of bright yellow polyethylene tape with 2" black letters with a CAUTION message.

BUCKET AND WRINGER (1 EACH). 10 gallon polypropylene bucket and wringer for use in final residue clean-up.

COTTON STRING MOP AND DISINFECTANT DETERGENT (1 EACH). Cotton string mop head, mop handle, and 32-ounce bottle of disinfectant detergent. Several mop heads and bottles of detergent should be kept in reserve.

POLYCARBONATE PICK UP TONGS (1 PAIR). Plier type tongs for hazardous debris pick up.

pH PAPER (1 ROLL). ½" wide x 50' roll of pH paper with color chart. One dip identifies pH from 0 to 14 with single color match.

EYEWASH BOTTLE (32 OUNCE). A portable, squeeze bottle type eyewash bottle filled with distilled water.

EMERGENCY FLASHLIGHT (1 EACH). A rechargeable 6-volt explosion-proof flashlight should be plugged in where the cart is stored and put on the cart when it is taken to a spill site.

TRAINING. Thorough operator training should be conducted on the hazards of spill response activity and requirements for personal protection including respirator use. Refresher training should be given not less than each six months. This should include dry-run, mock emergency response practice.

A spill response standard operating procedure should be developed and used by all emergency response personnel. It must be brief and clear, with one sentence instructions, and maintained on the cart at all times to be effective under emergency conditions.

A chemical or hazardous material properties handbook should be maintained as a ready reference on the cart.

A cart contents map should be used with the cart to identify response material storage locations. Color coding would also be useful.

Emergency phone numbers should be maintained on the cart.

An incident log and material use record book should be maintained to describe the spill incident and record the response and clean up supplies used. Analysis of these reports will provide valuable data concerning "problem" locations or materials, while also providing useful information on procedural changes needed to reduce similar spills in the future.

Include an inspection reporting procedure for cart operating condition and required supply of response contents. Inspections should occur every 30 days to assure optimum operating condition.

APPENDIX D

BIOSAFETY LEVELS APPROPRIATE FOR SPECIFIC AGENTS

The following recommendations are based upon the 3rd edition of the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 1993. Selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a number of factors that the principal investigator must take into account. Principal investigators should use the following as a guideline to assist in decision making.

ARBOVIRUSES AND RELATED ZOONOTIC DISEASES	CONTAINMENT RECOMMENDATIONS
West Nile Virus	BSL-2 practices, containment equipment and facilities are recommended for activities with human diagnostic specimens, although it is unusual to recover virus from specimens from clinically ill patients. BSL-2 is recommended for processing field collected mosquito pools whereas BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of WNV cultures and for experimental animal and vector studies, respectively. Dissection of field collected dead birds for histopathology and culture is recommended at BSL-3 containment due to the potentially high level of virus found in such samples. Non-invasive procedures performed on dead birds (such as oropharyngeal or cloacal swabs) can be conducted at BSL-2.
Eastern Equine Encephalitis (EEE) Virus Venezuelan Equine Encephalitis (VEE)Virus Western Equine Encephalitis (WEE)Virus	Diagnostic and research activities involving clinical materials, infectious cultures, and infected animals or arthropods should be performed under BSL-3 practices, containment equipment, and facilities. Due to the high risk of aerosol infection, additional personal protective equipment, including respirator protection, should be considered for non-immune personnel. Animal work with VEE virus, EEE virus, and WEE virus should be performed under ABSL-3. HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEE virus. Investigational vaccines for EEE virus, VEE virus and WEE virus may be available in limited quantities and administered on-site at the Special Immunization Program, USAMRIID.
Rift Valley Fever Virus (RVFV)	BSL-3 practices, containment equipment and facilities are recommended for processing human or animal materials in endemic zones or in non-endemic areas in emergency circumstances. Particular care should be given to stringent aerosol containment practices, autoclaving waste, decontamination of work area, and

	control of egress of materials from the laboratory. Other cultures, cells or similar biological material that could potentially harbor RVFV should not be used in a RVFV laboratory and subsequently removed. Diagnostic or research studies outside endemic areas should be performed in a BSL-3 laboratory. Personnel also must have additional respiratory protection (such as PAPR) or be vaccinated for RVFV. In addition, for research conducted in non-endemic areas, the USDA may require full BSL-3 Ag containment. An investigational vaccine for RVFV may be available in limited quantities and administered on-site at the Special Immunization Program, USAMRIID.
Vaccine strains of BSL-3 and BSL-4 arboviruses Chikungunya 181/25 Junin Candid #1 Rift Valley Fever MP-12 Venezuelan equine encephalomyelitis TC83 Yellow Fever 17-D Japanese encephalitis 14-14-2	May be handled as BSL-2 viruses
Arboviruses: Abras, Abu Hammad, Acado, Acara, Adelaide River, Aguacate, Aino, Alenquer, Alfuy, Almeirim, Almpiwar, Altamira, Amapari, Ambe, Ananindeua, Andasibe, Anhanga, Anhembi, Anopheles A, Anopheles B, Antequera, Apeu, Apoi, Aransas Bay, Arbia, Arboledas, Aride, Ariquemes, Arkonam, Armero, Aroa, Aruac, Arumateua, Arumowot, Aura, Avalon, Babahoyo, Babanki, Bagaza, Bahig, Bakau, Baku, Bandia, Bangoran, Bangui, Banzi, Barmah Forest, Barranqueras, Barur, Batai, Batama, Batken, Bauline, Bebaru, Belem, Belmont, Belterra, Benevides, Benfica, Berrimah, Beritoga, Bimbo, Bimitti, Birao, Bluetongue (non-exotic), Bobaya, Bobia, Boraceia, Botambi, Boteke,	BSL-2 practices, containment equipment and facilities are recommended for activities with potentially infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonate hen=s eggs, and rodents. Large quantities and/or high concentrations of any virus have the potential to overwhelm both innate immune mechanisms and vaccine-induced immunity. When a BSL-2 virus is being produced in large quantities, or in high concentrations, additional risk assessment is required. This might indicate BSL-3 practices, including additional respiratory protection, based on the risk assessment of the proposed experiment.

Bouboui, Bozo, Breu Branco, Buenaventura, Bujaru, Bunyamwera, Bunyip Creek, Burg El Arab, Bushbush, Bussuquara, Buttomwillow, Bwamba, Cacao, Cache Valley, Cacipacore, Caimito, Calchaqui, California Encephalitis, Calovo, Canancia, Candiru, Caninde, Cape Wrath, Capim, Caraipe, Carajas, Carapura, Carey Island, Catu, Chaco, Chagres, Chandipura, Changuinola, Charleville, Chenuda, Chilibre, Chim, Chobar Gorge, Clo Mor, Coastal Plains, Cocal, Codajas, Colorado Tick Fever, Connecticut, Corfou, Corriparta, Cotia, Cowbone Ridge, Csiro Valley, Cuiaba, Curionopolis, Dabakala, D=Aguilar, Dakar Bat Virus, Dengue Virus Type 1, Dengue Virus Type 2, Dengue Virus Type 3, Dengue Virus Type 4, Dera Ghazi Khan, Dhori, Durania, Edge Hill, Entebbe Bat, Epizootic Hemorrhagic Disease, Erve, Estero Real, Eubenangee, Eyach, Farmington, Flanders, Fomede, Forecariah, Fort Morgan, Fort Sherman, Frijoles, Gabek Forest, Gadgets Gully, Gamboa, Gan Gan, Garba, Getah, Gomoka, Gordil, Gossas, Grand Arbaul, Gray Lodge, Great Island, Guajara, Guama, Guaratuba, Guaroa, Gumbo Limbo, Gurupi, Hart Park, Hazara, Highlands J, Huacho, Hughes, Iaco, Ibaraki, Icoaraci, Ieri, Ife,)**:** Iguape, Ilesha, Ilheus, Ingwavuma, Inhangapi, Inini, Inkoo, Ippy, Iriri, Irituia, Isfahan, Israel Turkey Meningitis, Itacaiunas, Itaituba, Itaporanga, Itaqui, Itimirim, Itupiranga, Ixcanal, Jacareacanga, Jacunda,	

Jamanxi, Jamestown Canyon, Japanaut, Jari, Jatobal, Jerry Slough, Joa, Johnston Atoll, Joinjakaka, Juan Diaz, Jurga, Jurona, Juruaca, Jutiapa, Kadam, Kaeng Khoi, Kaikalur, Kairi, Kaisodi, Kamese, Kamiti River, Kammavanpettai, Kannamangalam, Kao Shaun, Karimabad, Karshi, Kasba, Kedougou, Kemerovo, Kern Canyon, Ketapang, Keterah, Keuraliba, Keystone, Khasan, Kimberley, Kindia, Kismayo, Klamath, Kokobera, Kolongo, Koongol, Kotonkan, Kowanyama, Kununurra, Kwatta, Kyzylagach, La Crosse, Lagos Bat, La Joya, Lake Clarenton, Landjia, Langat, Lanjan, Las Maloyas, Latino, Lebombo, Le Dantec, Lednice, Lipovnik, Llano Seco, Lokern, Lone Star, Lukuni, Macaua, Madrid, Maguari, Mahogany Hammock, Main Drain, Malakal, Manawa, Manitoba, Manzanilla, Mapputta, Maprik, Maraba, Marajo, Marco, Mariquita, Marituba, Marrakai, Matariya, Matruh, Matucare, Mayaro, Mboke, Meaban ,Melao, Mermet ,Middelberg, Minatitlan, Minnal, Mirim, Michell River, Modoc, Moju, Mojui Dos Campos, Mono Lake, Mont. Myotis Leukemia, Monte Dourado, Moriche, Morro Bay, Morumti, Mosqueiro, Mossuri, Mount Elgon Bat, M=Poko, Mucura, Munguba, Murutucu, Mykines, Naranjal, Nariva, Nasoule, Navarro, Ndelle, Ndumu, Neputo, Netivot, New Minto, Ngaingan, Ngoupe, Nique, Nkolbisson, Nodamura, Nola, Northway, Ntaya, Nugget, Nyamanini, Nyando, Oak Vale,	

Odrenisrou, Okhotskiy, Okola, Olifantsvlei, Omo, O=Nyong-Nyong, Oriboca, Oriximina, Orungo, Ossa, Ouango, Oubangui, Oubi, Ourem, Pacora, Pacui, Pahayokee, Palma, Palestina, Palyam, Para, Paramushir, Parana, Paroo River, Pata, Pathum Thani, Patois, Peaton, Perient, Petevo, Phnom-Phen Bat, Pichinde, Picola, Pixuma, Playas, Pongola, Ponteves, Polosi, Precarious Point, Pretoria, Prospect Hill, Puchong, Pueblo Viejo, Punta Salinas, Punta Toro, Purus, Qalyub, Quaranfil, Radi, Razdan, Resistencia, Restan, Rhode Island, Rio Bravo, R,io Grande Rio Preto, Rochambeau, Ross River, Royal Farm, Sabo, Saboya, Sagiyama, Saint-Floris, Sakhalin, Salanda, Salchabod, Salmon River, Sal Vieja, San Angelo, Sandfly Fever Naples, Sandfly Fever Sicilian, Sandjimba, Sango, San Juan, San Perlita, Santarem, Santa Rosa, Saraca, Sathuperi, Saumarez Reef, Sawgrass, Sebokele, Sedlec, Seletar, Sembalam, Sena Madureira, Sepik, Serra Do Navio, Serra Norte, Shamonda, Shark River, Shokwe, Shuni, Silverwater, Simbu, Simian Hemorrhagic Fever, Sindbis, Sixgun City, Snowshoe River, Sokoluk, Soldado, Sororoca, Spondweni, Stratford, Sunday Canyon, Tacaiuma, Tacaribe, Taggert, Tahyna, Tai, Tamdy, Tamiami, Tanga, Tanjong Rabok, Tapara, Tataguine, Tehran, Telok Forest, Tembe, Tembusu, Tensaw, Termeil, Tete, Thiafora, Thimin, Thogoto, Thottapalayam, Tibrogargan, Tilligerry, Timbo, Timboteua, Tinaroo, Tindholmur, Tlacotalpan, Toscana, Toure,	

Tracambe, Tribec, Triniti, Trivittatus, Trocara, Trombetas, Trubanaman, Tsurusa, Tucurui, Tula, Tunis, Turlock, Turuna, Tyuleniy, Uganda S, Umatille, Umbre, Una, Upolu, Uriurana, Urucuri, Usutu, Utinga, Uukuniemi, Vellore, Venkatapuram, Vinces, Virgin River, Vesticular Stomatitis Alagoas, Vesticular Stomatitis Indiana, Vesticular Stomatitis New Jersey, Wad Medani, Wallal, Wanowrie, Warrego, Whatoroa, Witwatersrand, Wongal, Wongorr, Wyeomyia, Xiburema, Yacaaba, Yaounde, Yaquina Head, Yata, Yogue, Yoka, Yug Bogdanovac, Zaliv Terpeniya, Zegla, Zika, Zirqa	
Arboviruses: African Horsesickness, African swine fever, Akabane, Allpahuayo, Andes, Araguari, Bear Canyon, Bermejo, Bhanja, Bluetoungue (exotic serotypes), Bovine Ephemeral Fever, Cabassou, Cano Delgadito, Chukugunga, Deer Tick Fever, Dobrava-Belgrade, Doouglas, Dugbe, Eastern Equine Encephalitis, Enseada, Everglades, Flexal, Garissa, Germiston, Hantaan, Issyk-Kul, Japanese Encephalitis, Khabarovsk, Koutango, Kunjin, Laguna Negra, Lechiguanas, Louping Ill, Maporal, Mobala, Mopeia, Mucamto, Murray Valley Encephalitis, Nairobi Sheep Disease, Negishi, Ngari, Oran, Oropouche, Pergamino, Pirital, Piry, Powassan, Puumala, Rift Valley Fever, Rocio, Saaremaa, Semliki Forest, Seoul, Sin Nombre, Slovakia, Somone, Sripur, St. Louis Encephalitis, Tonate, Topografov, Venezuelan Equine Encephalitis, Wesselsbron,	BSL-3 practices, containment equipment and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animal or arthropods. Situations may arise for which enhancements to BSL-3 practices and equipment are required. An example would be when a BSL-3 laboratory performs diagnostic testing on specimens from patients with hemorrhagic fever thought to be due to dengue or yellow fever viruses. When the origin of these specimens is Africa, the Middle East, or South America, such specimens might contain etiologic agents that are usually manipulated in a BSL-4 facility. Examples of enhancements to BSL-3 laboratories might include 1) enhanced respiratory protection of personnel against aerosols; 2) HEPA filtration of dedicated exhaust air from the laboratory; 3) personal body shower. Additional appropriate training for all animal care personnel should be considered.

Western Equine Encephalitis, West Nile, Whitewater Arroyo, Xingu, Yellow fever	
Arboviruses: Absettarov, Alkhumra, Congo-Crimran Hemorrhagic Fever, Ebola (including Reston), Guanarito, Hanzalova, Hypr, Junin, Kumlinge, Kyasanur Forest Disease, Lassa, Machupo, Marburg, Omsk Hemorrhagic Fever, Russian Spring-Summer Encephalitis, Sabia,	BSL-4 practices, containment equipment and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal or arthropod origin. Clinical specimens from persons suspected of being infected with one of the agents listed should be submitted to a laboratory with a BSL-4 maximum containment facility. Junin virus has been reclassified to BSL-3 provided that all at-risk personnel are immunized and the laboratory is equipped with HEPA-filtered exhaust. Abbsettarov, Hanzalova, Hypr, and Kumlinge viruses have been reclassified to BSL-3 provided all at-risk personnel are vaccinated with Central European Tick-borne Encephalitis (CETBE) viruses vaccine.

BACTERIAL AGENTS	CONTAINMENT RECOMMENDATIONS
Bacillus anthracis	BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. ABSL-2 practices, containment equipment, and facilities are recommended for studies utilizing experimentally infected laboratory rodents. BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. Workers who frequently centrifuge *B. anthracis* suspensions should use autoclavable aerosol-tight rotors. In addition, regular routine swabbing specimens for culture should be routinely obtained inside the rotor and rotor lid and, if contaminated, rotors should be autoclaved before re-use.
Bordetella pertussis	BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical material and cultures. ABSL-2 practices and containment equipment should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or

	safety centrifuges should be used for activities likely to generate potentially infectious aerosols. BSL-3 practices, containment equipment and facilities are appropriate for production operations.
Brucella spp. *B. melitensis* *B. suis* *B. abortus* *B. canis* *B. maris*	BSL-2 practices, containment equipment, and facilities are recommended for routine clinical specimens of human or animal origin. Products of conception containing or believed to contain pathogenic *Brucella* should be handled with BSL-3 practices due to the high concentration of organisms per gram of tissue. BSL-2 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of cultures of pathogenic *Brucella* spp. listed and for experimental animal studies
Burkholderia mallei	Primary isolations from patient fluids or tissues may be performed with BSL-2 practices, containment equipment, and facilities in a BSC. Procedures must be performed under BSL-3 containment whenever infectious aerosols or droplets are generated, such as during centrifugation or handling infected animals or when large quantities of the agent are produced. Procedures outside of a BSC (centrifugation, animals manipulation, etc.) that generate infectious aerosols require respiratory protection. Sealed cups should be used with all centrifuges and these should be opened only inside a BSC. Gloves should be worn when working with potentially infectious material or animals. Animal work with *B. mallei* should be dome with ABSL-3 practices, containment equipment, and facilities.
Burkholderia pseudomallei	Work with clinical specimens from patient suspected of having melioidosis and of *B. pseudomallei* cultures may be perform with BSL-2 practices, containment equipment, and facilities. Work should be done in a BSC. Gloves should always be worn when manipulating the organism. In case where infectious aerosols or droplets could be produced, or where production quantities of the organism are generated, these procedures should be confined to BSL-3 facilities with all the pertinent primary containment against escape of aerosols. Respiratory protection must be used if the organism is manipulated outside a BSC, such as during centrifugation or handling infected animals. Sealed cups should be used on all centrifuges and these should be opened only in a BSC. Animal studies with this agent should be done at ABSL-3.

Campylobacter spp.	BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.
Chlamydia psittaci *Chlamydia trachomatis* *Chlamydia pneumoniae*	BSL-2 practices, containment equipment, and facilities are recommended for personnel working with clinical specimens and cultures or other materials known or suspected to contain the ocular genital serovars (A through K) of *C. trachomatis* or *C. pneumoniae*. BSL-3 practices, containment equipment, and facilities are recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin. Wetting the feather of infected birds with a detergent-disinfectant prior to necropsy can appreciably reduce the risk of aerosols of infected feces and nasal secretions on the feathers and external surfaces of the bird. Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed, including but not limited to the use of primary containment equipment such as BSCs. BSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds. BSL-3 practices and containment equipment are recommended for activities involving work with culture specimens or clinical materials known to contain or be potentially infected with the LGV serovars (L_1 through L_3) of *C. trachomatis*. Laboratory work with the LGV serovars of *C. trachomatis* can be conducted in a BSL-2 facility as long as BSL-3 practices are followed when handling potentially infectious materials, including, but not limited to, use of primary containment equipment such as BSCs. Gloves are recommended for the necropsy of birds and mice, the opening of inoculated eggs, and when there is the likelihood of direct skin contact with infected tissues, bubo fluids, and other clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with animals that have been experimentally infected with genital serovars of *C. trachomatis* or *C. pneunomoniae*. BSL-3 practices, containment equipment, and facilities

	are indicated for activities involving any of these species with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.
Clostridium botulinum	BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin including the handling of potentially contaminated food. Solutions of 0.1% sodium hypochlorite or 0.1N sodium hydroxide readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate. BSL-3 practices, containment equipment, and facilities are required for activities with a high potential for aerosol or droplet production, and for those involving large quantities of the organism or of the toxin. ABSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.
Clostridium tetani and Tetanus toxin	BSL-2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures or toxin. ABLS-2 practices, containment equipment, and facilities are recommended for animal studies.
Corynebacterium diphtheriae	BSL-2 practices, containment equipment, and facilities are recommended all for activities utilizing known or potentially infected clinical materials or cultures. ABLS-2 practices, containment equipment, and facilities are recommended for studies utilizing infected laboratory animals.
Francisella tularensis	BSL-2 practices, containment equipment, and facilities are recommended for activities involving clinical materials of human or animal origin suspected or known to contain *F. tularensis*. Laboratory personnel should be informed of the possibility of tularemia as a differential diagnosis when samples are submitted for diagnostic tests. BSL-3 and ABSL-3 practices, containment equipment and facilities are recommended for all manipulations of suspect cultures, animal necropsies and for experimental animal studies. Preparatory work on culture or contaminated materials for automated identification systems should be performed at the BSL-3. Characterized strains of reduced virulence such as *F. tularensis* Type B (strain LVS) and *F. tularensis* subsp. *novicida* (strain U112) can be manipulated in BSL-2. Manipulation of reduced

	virulence strains at high concentrations should be conducted using BSL-3 practices.
Helicobacter spp.	BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially contain the agent. ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimental or naturally infected animals.
Legionella pneumophilia and other Legionella-like Agents	BSL-2 practices, containment equipment, and facilities are recommended for activities involving the use or manipulation of potentially infectious material, including minimizing the potential for dissemination of the organism from cultures of organisms known to cause disease. ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimentally infected animals. Routine processing of environmental water samples for Legionella may be performed with standard BSL-2 practices. For activities likely to produce extensive aerosols and when large quantities of the pathogenic organism are manipulated, BSL-2 with BSL-3 practices is recommended.
Leptospira spp.	BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infected tissues, body fluids, and cultures. The housing and manipulation of infected animals should be performed in ABSL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.
Listeria monocytogenes	BSL-2 practices, containment equipment, and facilities are recommended when working with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn when handling infected or potentially infected materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, pregnant women should be advised of the risk of exposure to *L. monocytogenes.*
Mycobacterium leprae	BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. Extraordinary care should be taken to avoid accidental parenteral inoculation with contaminated

	sharp instruments. ABSL-2 practices, containment equipment, and facilities are recommended for all animal studies utilizing rodents armadillos, and NHP, because coughing with dissemination of infectious droplets does not occur in these species.
Mycobacterium tuberculosis complex	BSL-2 practices, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol- generating activities must be conducted in a BSC. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. Liquefaction and concentration of sputa for acid-fast staining may be conducted safety on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution and waiting 15 minutes before processing. BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of culture of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Animal studies using guinea pigs or mice can be conducted at ABSL-2. BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. Manipulations of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guerin (BCG) can be performed at BSL-2 in laboratories that do not culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of as appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria.
Mycobacterium spp. (Other)	BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacteria* spp. Other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and care must be exercised to the correct identification of cultures. Special caution should be exercised in handling *M. ulcerans* to avoid skin exposure. ABSL-2 practices, containment

	equipment, and facilities are recommended for animal studies. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria.
Neisseria gonorrhoeae	BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical material or cultures. Gloves should be worn when handling infected laboratory animals and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions such as those described for BSL-3 may be indicated when there is a high risk of aerosol or droplet production, and for activities involving production quantities or high concentration of infectious materials. Animal studies may be perform at ABSL-2
Neisseria meningitidis	Specimens for *N. meningitidis* analysis and cultures of *N. m*eningitidis not associated with invasive disease may be handled in BSL-2 facilities with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of *N. meningitidis* should be manipulated within a BSC. Isolates of unknown source should be treated as sterile-site isolates. If a BSC is unavailable, manipulation of these isolates should be minimized, primarily focused on serogroup identification using phenolized saline solution while wearing laboratory coat, gloves, and safety glasses or full face splash shield. BSL-3 practices and procedures are indicated for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. Animal studies should be performed under ABSL-2 conditions.
Salmonella serotypes (not *S. typhi*)	Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination

	of clean hands or the use of sinks equipped with remote control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.
Salmonella typhi	Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of clean hands or the use of sinks equipped with remote control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of organisms. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals. ABSL-3 conditions may be considered for protocols involving aerosols.
Shiga toxin (Verocytotoxin)-producing *Escherichia coli*	Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of clean hands or the use of sinks equipped with

	remote control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally or naturally infected animals.
Shigella	Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of clean hands or the use of sinks equipped with remote control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally or naturally infected animals.
Treponema pallidum	BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or other clinical samples from humans or infected rabbits. Glove should be worn when there is a likelihood of direct skin contact with infective materials. Periodic serological monitoring should be considered in personnel regularly working with these materials. ABSL-2 practices, containment equipment, and facilities are recommended for work with infected animals.
Vibrio enteris species	BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.
Yersinia pestis	BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the

	handling of potentially infectious clinical materials and cultures. In addition, because the infectious dose is so small, all work, including necropsies of potentially infected animals should be performed in a BSC. Special care should be taken to avoid generating aerosols or airborne droplets while handling infectious materials or while performing necropsies on naturally or experimentally infected animals. Gloves should be worn when handling potentially infectious materials including field or laboratory infected animals. BSL-3 is recommended for activities with high potential for droplet or aerosol production, and for activities involving large scale production or high concentrations of infectious materials. Resistance of *Y. pestis* strains to antibiotics used in the treatment of plague should be considered in a thorough risk assessment and may require additional containment or personal protective equipment. For animal studies, a risk assessment that takes into account the animal species, infective strain, and proposed procedures should be performed in order to determine if ABSL-2 or ABSL-3 practices, containment equipment, and facilities should be employed. BSL-3 facilities and arthropod containment level 3 practices are recommended for all laboratory work involving infected arthropods.

FUNGAL AGENTS	CONTAINMENT RECOMMENDATIONS
Blastomyces dermatitidis	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials, animal tissues, yeast-form cultures, and infected animals. BSL-3 practices, containment equipment and facilities are required for handling sporulating mold-form cultures already identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia.
Coccidiodies immitis *Coccidiodies posadasii*	BSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues. ABSL-2 practices, containment equipment, and facilities are recommended for experimental animal studies when the route of challenge is parenteral. BSL-3 practices, containment equipment, and facilities, are recommended for propagating and manipulating sporulating cultures already identified as *Coccidiodies* spp. and for processing soil or other environmental materials known to contain infectious

	arthroconidia. Experimental animal studies should be done at ABSL-3 when challenge is via the intranasal or pulmonary route.
Cryptococcus neoformans	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with known or potentially infectious clinical, environmental, or culture materials and with experimental infected animals. This agent and any samples that may contain this agent should also be handled in a Class II BSC.
Histoplasma capsulatum	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, animal tissues and mold cultures, identifying cultures in routine diagnostic laboratories, and for inoculating experimental animals, regardless of route. Any culture identifying dimorphic fungi should be handled in a Class II BSC. BSL-3 practices, containment equipment, and facilities, are recommended for propagating and sporulating cultures of *H. capsulatum* in the mold form as well as processing soil or other environmental materials known or likely to contain infectious conidia.
Sporothrix schenckii	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of suspected clinical specimens, soil and vegetation, and experimental animal activities with *S. schenckii*. Gloves should be worn during manipulation of *S. schenckii* and when handling experimentally infected animals. Any culture identifying dimorphic fungi should be handled in a Class II BSC.
Dermatophytes *Epidermophyton* spp. *Microsporum* spp. *Trichophyton* spp.	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling cultures and soil samples. Any culture identifying dimorphic fungi should be handled in a Class II BSC.
Miscellaneous Molds *Penicillium marneffei* *Bipolaris* spp. *Cladophialophora bantiana* *Exophiala (Wangiella) dermatitidis* *Exserohilum* spp. *Fonsecaea pedrosoi* *Ochroconis gallopava* *(Dactylaria gallopava)* *Ramichloridium mackenziei* *(Ramichloridium obovoideum)*	BSL-2 practices, containment equipment, and facilities, are recommended for propagating and manipulating cultures known to contain these agents. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Rhinocladiella atrovirens *Scodosporium*	

PARASITIC AGENTS	CONTAINMENT RECOMMENDATIONS
Blood & Tissue Protozoal Parasites *Leishmania* spp. *Plasmodium* spp. *Toxoplasma gondii* *Trypanosoma* spp. *Babesia microti* *Microsporidia* (actually fungal)	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed. Infected arthropods should be maintained in facilities that reasonably preclude the exposure of personnel or the escape of insects. Personal protection (e.g., lab coat, gloves, face shield), in conjunction with containment in a BSC, is indicated when working with cultures, tissue homogenates, or blood containing organisms.
Intestinal Protozoal Parasites *Cryptosporidia* spp. *Isospora belli* *Entamoeba histolytica* *Giardia* spp.	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed. Primary containment (e.g., BSC) or personal protection (e.g., face shield) is especially important when working with *Cryptosporidium*. Oocysts are infectious when shed, often are present in stool in high numbers, and are environmentally hardy.
Trematode Parasites *Fasciola hepatica* *Schistosoma* spp.	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed. Gloves should be worn when there may be direct contact with water containing cercariae or vegetation with encysted metacercariae from naturally or experimentally snail intermediate hosts. Long-sleeved laboratory coats or other protective garb should be worn when working in the immediate area of aquaria or other water sources that may contain schistosome cercariae. Water from laboratory aquaria containing snails and cercariae should be decontaminated before discharged to sanitary sewers.
Cestode Parasites *Echinococcus* spp. *Hymenolepis nana* *Taenia solium*	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed. Special attention should be given to personnel hygiene (e.g., hand washing) and laboratory practices that would reduce the risk of accidental ingestion of infective eggs. Gloves are recommended when there may be direct contact with feces or with surfaces contaminated with fresh feces of carnivores infected with *Echinococcus* spp., humans infected with *T. solium*. Or humans or rodents infected with *H. nana*.

Nematode Parasites *Ancylostoma* spp. *Ascaris lumbricoides* *Enterobius vermicularis* *Strongyloides* spp. *Trichinella* spp. *Toxocara* spp. *Baylisascaris* spp.	BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the nematodes listed. Exposure to aerosolized sensitizing antigens of ascarids should be avoided. Primary containment (e.g., BSC) is recommended for work that may result in aerosolization of sensitization antigens.

PRION DISEASES	CONTAINMENT RECOMMENDATIONS
Human Prion Diseases Kuru Creutzfeldt-Jacobs disease (CJD) Sporatic CJD (sCJD) Variant CJD (vCJD) Familial CJD (fCJD) Iatrogenic CJD (iCJD) Gerstmann-Straussler-Scheinker syndrome (GSS) Fatal Familial Insomnia (FFI) Animal Prion Diseases Scrapie Bovine Spongiform Encephalopathy (BSE) Chronic Wasting Disease (CWD) Exotic Ugulate Encephalopathy (EUE) Feline Spongiform Encephalopathy Transmissible Mink Encephalopathy (TME)	In the laboratory setting, prions from human tissues and human prions propagated in animals should be manipulated at BSL-2. Animal prions are considered BSL-2 pathogens. Due to the high probability that BSE prions have been transmitted to humans, certain circumstances may require the use of BSL-3 facilities. However, when a prion from one species is inoculated into another, the resultant infected animal should be treated according to the guidelines applying to the source of infection. Routine autopsies and the processing of small amounts of formalin-fixed tissues containing prions can safety be done using BSL-2 precautions. When performing necropsies on large animals where there is an opportunity that the worker may accidentally be splashed or have contact with high-risk materials (e.g., spinal column, brain, etc.)personnel should wear full body personal protective equipment (e.g., gloves, rear closing gown and face shield). Disposable plasticwear, which can be discarded as a dry regulated medical waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 1N NaOH and rinsed with water. HEPA filters should be bagged out and incinerated. It is prudent to avoid the generation of aerosol or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that impervious gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids.

RICKETTSIAL AGENTS	CONTAINMENT RECOMMENDATIONS
Coxiella burnetti	BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears. BSL-3 practices and facilities are

	recommended for activities involving the inoculation, incubation, and harvesting of embryonated eggs or cell culture, the necropsy of infected animals and the manipulation of infected tissue. Experimentally infected animals should be maintained under ABSL-3 because infected rodents may shed the organism in urine or feces. A specific plaque-purified clonal isolate of an avirulent (Phase II) strain (Nine Mile) may be safely handled under BSL-2 conditions.
Rickettsia prowazekii *Rickettsia typhi (R. mooseri)* *Orientia (Rickettsia) tsutsugamushi* Spotted Fever Group Agents *Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica, Rickettsia japonicum*	BSL-2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures, and for the staining of impression smears. BSL-3 practices, containment equipment and facilities are recommended for all other manipulations of known or potentially infectious materials, including necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or cell culture. ABSL-2 practices, containment equipment, and facilities are recommended for the holding of experimentally infected animals other than arthropods. BSL-3 practices, containment equipment, and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease. Several species, including *R. montana, R. rhipicephali, R. belli,* and *R. canada,* are not known to cause human disease and may be handled under BSL-2 conditions. New species are being described frequently and should be evaluated for appropriate containment on a case-by-case basis. Because of the proven value of antibiotic therapy in the early stages of rickettsial infection, it is essential that laboratories have an effective system for reporting febrile illnesses in laboratory personnel, medical evaluation of potential cases and, when indicated, institution of appropriate antibiotic therapy.

TOXIN AGENTS	CONTAINMENT RECOMMENDATIONS
Botulnum Neurotoxin (BoNT)	BSL-2 practices, containment equipment, and facilities are recommended for routine dilutions, titrations, or diagnostic studies with materials known to contain or have the potential to contain BoNT. Additional primary containment and personnel precautions, such as those recommended for BSL-3 should be

	implemented for activities with high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of toxin. Non-immunized personnel should be discouraged from entering the laboratory when BoNT is in use until after the toxin and all work surfaces have been decontaminated. Purified preparations of toxin components, e.g. isolated BoNT Alight chains@ or Aheavy chains@ should be handled as if contaminated with holotoxin unless proven otherwise by toxicity bioassays.
Staphylococcal Enterotoxins (SE)	BSL-2 practices, containment equipment and facilities should be used when handling SE or potentially contaminated materials. Because SE is highly active by the oral or ocular exposure route, the use of a laboratory coat, gloves and safety glasses is mandatory when handling toxin or toxin-contaminated solutions. Frequent and careful hand-washing and laboratory decontamination should be strictly enforced when working with SE. Depending upon a risk assessment of the laboratory operation, the use of a disposable face mask may be required to prevent accidental ingestion. BSL-3 facilities, equipment, and practices are indicated for activities with a high potential for aerosol or droplet production or those involving the use of large concentrations of SE.
Ricin Toxin	BSL-2 practices, containment equipment and facilities are recommended, especially a laboratory coat, gloves, and protective mask, when handling ricin toxin or potentially contaminated material. Ricin is a relatively non-volatile cytotoxin and irritant that should be handled in the laboratory as a non-volatile toxic chemical. A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with an exhaust HEPA filter and charcoal filter are indicated for activities with a high potential for aerosol, such as powder samples, and the use of large quantities of toxin. Laboratory coat, gloves, and a full face respirator should be worn if there is potential for creating a toxin aerosol.
Low Molecular Weight (LMW) Toxins such as T-2 mycotoxin Saxitoxin Tetrodotoxin Brevetoxin	When handling LMW toxins or potentially contaminated material, BSL-2 practices, containment equipment and facilities are recommended, especially the wearing of a laboratory coat, safety glasses, and disposable gloves; the gloves must be impervious to organic solvents or other diluents employed with the

Palytoxin Conotoxins (α-GI & α-MI) Microcystin-LR (cyanoginosins)	toxin. A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with an exhaust HEPA filter and charcoal filter are indicated for activities with a high potential for aerosol, such as powder samples, and the use of large quantities of toxin. Laboratory coat and gloves should be worn if potential skin contact exists. The use of a protective mask should be considered of potential aerosolization of toxin exists. For LMW toxins that are not easily decontaminated with bleach solutions, it is recommended to use pre-positioned , disposable liners for laboratory bench surfaces to facilitate clean up and decontamination.

VIRAL AGENTS	CONTAINMENT RECOMMENDATIONS
Hantaviruses	BSL-2 practices, containment equipment and facilities are recommended for laboratory handling of sera from persons potentially infected with hantaviruses. The use of a certified BSC is recommended for all handling of human body fluids when potential exists for spatter or aerosol Potentially infectious tissue samples should be handled in BSL-2 facilities following BSL-3 practices and procedures. Cell-culture virus propagation and purification should be carried out in a BSL-3 facility with BSL-3 practices, containment equipment and procedures. Experimentally infected rodent species known not to excrete the virus can be housed in ABSL-2 facilities using ABSL-2 practices and procedures. Primary physical containment devices including BSCs should be used whenever procedures with potential for producing aerosol are conducted. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using BSL-3 practices, containment equipment and procedures. All work involving inoculation of virus-containing samples into rodent species permissive for chronic infections should be conducted at ABSL-4.
Hendra Virus (formerly known as Equine Morbillivirus) Nipah Virus	Because of the unknown risk to laboratory workers and the potential impact on indigenous livestock should the virus escape a diagnostic or research laboratory, health officials and laboratory managers should evaluate the need to work with the virus and the containment capability of the facility before undertaking any work with Hendra, Nipah, or suspected related viruses. BSL-4 is required for all work with these viruses. Once a diagnosis of Nipah or Hendra virus is suspected, all diagnostic specimens also must be handled at BSL-4.

	ABSL-4 is required for any work with infected animals.
Hepatitis A Virus Hepatitis E Virus	BSL-2 practices, containment equipment and facilities are recommended for the manipulation of hepatitis A and E virus, infected feces, blood or other tissues. ABSL-2 practices and facilities are recommended for activities using naturally or experimentally infected nonhuman primates or other animal models that may shed the virus.
Hepatitis B Virus Hepatitis C Virus (formerly known as nonA nonB Virus) Hepatitis D Virus	BSL-2 practices, containment equipment and facilities are recommended for all activities utilizing known or potentially infectious body fluids or tissues. Additional primary containment and personnel precautions, such as those described for BSL-3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other NHP. Gloves should be worn when working with infected animals and when there is the likelihood of skin contact with infectious materials. In addition to these recommended precautions, persons working with HBV, HCV or other blood-borne pathogen should consult the OSHA Bloodborne Pathogens Standard.
Human Herpes Virus	BSL-2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAI, it is prudent to avoid the generation of aerosols during the handling of clinical materials of isolates, or during the necropsy of animals. Primary containment devices (e.g. BSCs) should be utilized to prevent exposure of workers to infectious aerosols. Additional containment and procedures, such as those described for BSL-3, should be considered when producing, purifying, and concentrating human herpes viruses, based on the risk assessment.
Influenza (contemporary)	BSL-2 facilities, practices, and procedures are recommended for diagnostic, research and production activities utilizing contemporary, circulating human influenza strains (e.g. H1/H3/B) and low pathogenicity avian influenza (LPAI) strains (e.g., H1-4, H6, H8-16), and equine and swine influenza viruses. ABSL-2 is

	appropriate for work with these viruses in animal models. All avian and swine influenza viruses require an APHIS permit. Based on economic ramifications and source of the virus, LPAI H5 and H7 and swine influenza viruses may have additional APHIS permit-driven containment requirements and personnel practices and/or restrictions.
Non-contemporary Human Influenza (H2N2) Strains	BSL-3 and ABSL-3 practices, procedures, and facilities are recommended with rigorous adherence to additional respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators or positive air-purifying respirators (PAPS) are recommended for use. Cold-adapted live attenuated H2N2 vaccine strains may continue to be worked with at BSL-2.
1918 Influenza Strain	BSL-3 and ABSL-3 practices, procedures, and facilities are recommended with rigorous adherence to additional respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators or positive air-purifying respirators (PAPS) are recommended for use. Large laboratory animals such as NHP should be housed in primary barrier systems in ABSL-3 facilities. Exhaust air should be HEPA-filtered. Personnel practices should be amended to include personal showers prior to exiting the laboratory.
Highly Pathogenic Avian Influenza (HPAI)	BSL-3 and ABSL-3 practices, procedures, and facilities are recommended with rigorous adherence to additional respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators or positive air-purifying respirators (PAPS) are recommended for use. Loose-housed animals infected with HPAI strains must be contained within BSL-3-Ag facilities. An APHIS permit is required and may carry additional containment requirements and personnel practices and/or restrictions.
Lymphocytic Choriomeningitis Virus	BSL-2 practices, containment equipment and facilities are suitable for activities utilizing known or potentially infectious body fluids, and for cell culture passage of laboratory-adapted strains. BSL-3 is required for activities with high potential for aerosol production, work with production quantities or high concentrations of infectious materials, and for manipulations of infected transplantable tumors, field isolates and clinical materials from human cases. Strains of LCMV that are shown to be lethal in nonhuman primates

	should be handled at BSL-3. ABSL-2 practices, containment equipment. And facilities are suitable for studies in adult mice with strains requiring BSL-2 containment. Work with infected hamsters also should be done at ABSL-3.
Poliovirus	BSL-2 practices, containment equipment and facilities are recommended fro all activities utilizing wild poliovirus infectious culture fluids, environmental samples, and clinical materials, in addition, potentially infectious materials collected for any purpose should be handled at BSL-2. Laboratory personnel working with such materials must have documented polio vaccination. Persons who have had a primary series of OPV or IPV and who are at an increased risk can receive another dose of IPV, but available data does not indicate the need for more than a single lifetime IPV booster for adults. ABSL-2 practices, containment equipment and facilities are recommended for studies of virulent viruses in animals. Laboratories should use authentic Sabin OPV attenuated strains unless there are strong scientific reasons for working with wild polioviruses. In anticipation of polio eradication, the WHO recommends destruction of all poliovirus stocks and potentially infectious materials if there is no longer a programmatic or research need for such materials. Institutions/laboratories in the United States that currently retain wild poliovirus infectious or potentially infectious material should be on the United States National Inventory maintained by the CDC. When one year has elapsed after detection of the last wild poliovirus worldwide, CDC will inform relevant institutions/laboratories about additional containment procedures. Safety recommendations are subject to change based on international poliovirus eradication activities.
Poxviruses	Worldwide, all live variola virus work is to be done only within WHO approved BSL-4/ABSL-4 facilities; one is at the CDC in Atlanta and the other is at the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia.
Rabies Virus (and related lyssaviruses)	BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals. Pre-exposure rabies vaccination is recommended for all individuals prior to working with lyssaviruses or infected animals, or engaging in

	diagnostic, production, or research activities with these viruses. Rabies vaccination is also recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used. Prompt administration of postexposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines. For routine diagnostic activities, it is not always feasible to open the skull or remove the brain of an infected animal in a BSC, but it is pertinent to use appropriate methods and personal protection equipment, including dedicated laboratory clothing, heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments, and a face shield or PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets. If a Stryker saw is used to open the skull, avoid contacting brain tissue with the blade of the saw. Additional primary containment and personnel precautions, such as those described for BSL-3, are indicated for activities with a high potential for droplet or aerosol production, and for activities involving large production quantities or high concentrations of infectious materials.
Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV)	BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids, and tissues. HTLV-1 and HTLV-2 should also be handled at this level. Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BSL-2 facility using BSL-3 practices. Activities involving large-scale volumes or preparation of concentrated HIV or SIV are conducted at BSL-3. ABSL-2 is appropriate for NHP and other animals infected with HIV or SIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2. In addition, persons working with HIV or SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.
SARS coronavirus	In clinical laboratories, whole blood, serum plasma and urine specimens should be handled using Standard Precautions which include the use of gloves, gown, mask and eye protection. Any procedure with the

	potential to produce aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a BSC. Use sealed centrifuge rotors or gasketed safety carriers for centrifugation. Rotors and safety carriers should be loaded and unloaded in a BSC. Procedures conducted outside a BSC must be performed in a manner that minimizes the risk of personnel exposure and environmental release. The following procedures may be conducted in the BSL-2 setting: pathologic examination and processing of formalin-fixed or otherwise inactivated tissue, molecular analysis of extracted nucleic acid preparations, electron microscopic studies with gluteraldehyde-fixed grids, routine examination of bacterial and fungal cultures, routine staining and microscopic analysis of fixed smears, and final packaging of specimens for transport to diagnostic laboratories for additional testing (specimens should already be in a sealed, decontaminated primary container) Activities involving manipulation of untreated specimens should be performed in BSL-2 facilities following BSL-3 practices. In the rare event that a procedure or process involving untreated specimens cannot be conducted in a BSC, gloves, gown, eye protection, and respiratory protection (acceptable methods of respiratory protection include: a properly-fitted, NIOSH-approved filter respirator (N-95 or higher level) or a PAPR equipped with HEPA filters) should be used. All personnel that may use respiratory protective devices should be enrolled in an appropriately constituted respiratory protection program. Work surfaces should be decontaminated upon completion of work with appropriate disinfectants. All waste must be decontaminated prior to disposal. SARS-CoV propagation in cell culture and the initial characterization of viral agents recovered in cultures of SARS specimens must be performed in a BSL-3 facility using BSL-3 practices and procedures. Risk assessment may dictate the additional use of respiratory protection. Inoculation of animals for potential recovery of SARS-CoV from SARS samples, research studies and protocols involving animal inoculation for characterization of putative SARS agents must be performed in ABSL-3 facilities using ABSL-3 work practices. Respiratory protection should be used as warranted by risk assessment. In the event of

	any break in laboratory procedure or accidents (e.g. accidental spillage of material suspected of containing SARS-CoV), procedures for emergency exposure management and/or environmental decontamination should be immediately implemented and the supervisor should be notified. The worker and the supervisor, in consultation with occupational health or infection control personnel, should evaluate the break in procedure to determine if an exposure occurred.

APPENDIX E

HHS AND USDA SELECT AGENTS AND TOXINS
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS

Abrin
Cercopithecine Herpesvirus 1 (Herpes Virus B)
Coccidioides posadasii
Conotoxins
Crimean-Congo haemorrhagic fever Virus
Diacetoxyscirpenol
Ebola Virus
Lassa fever Virus
Marburg Virus
Monkeypox Virus
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza Virus)
Ricin
Rickettsia prowazekii
Rickettsia rickettsii
Saxitoxin
Shiga-like ribosome inactivating proteins
South America Haemorrhagic Fever viruses
Guanarito
Flexal
Junin
Machupo
Sabia
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses
Central European Tick-borne encephalitis
Far Eastern Tick-borne encephalitis
Kyansanur Forest Disease
Omsk Hemorrhagic Fever
Russian Spring & Summer encephalitis
Variola major virus (Smallpox virus) and
Variola minor virus (Alastrim)
Yersinia pestis

USDA PLANT PROTECTION AND QUARANTINE SELECT AGENTS AND TOXINS

Candidatus Liberobacter *aficanus*
Candidatus Liberobacter *asiaticus*
Peronoschlerospora philippinensis
Ralstonia solanacearum race 3, biovar 2
Sclerophthora rayssiae var.*zeae*
Synchytrium endobioticum
Xanthomonas oryzae, pv *oryzicola*
Xylella fastidiosa (citrus variegated chlorosis strain)

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (Exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine flu virus
Cowdria ruminantium (Heartwater)
Foot-and-mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
(Alcelaphine herpesvirus type 1)
Menangle virus
Mycoplasma capricolum/M.F38/*M.mycoides Capri* (contagious caprine pleuropneumonia)
Mycoplasma mycoides mycoides (contagious bovine pleuropneumonia)
Newcastle disease virus (velogenic)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesticular disease virus
Vesticular stomatitis virus (Exotic)

OVERLAP SELECT AGENTS AND TOXINS

Bacillus anthracis
Botulinum neurotoxins
Botulinum Neurotoxin producing species of *Clostridium*
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei
Burkholderia pseudomallei
Clostridium perfringens epsilon toxin
Coccidioides immitis
Coxella burnetti
Eastern Equine Encephalitis virus
Francisella tularensis
Henrda virus
Nipah virus
Rift Valley fever virus
Shigatoxin
Staphylococcal enterotoxins
T-2 toxin
Venezuelan Equine Encephalitis Virus

APPENDIX F

HAZARDOUS WASTE - P-LISTED CHEMICALS

Acetaldehyde, chloro-
Acetamide, N-(aminothioxomethyl)-
Acetamide, 2-fluoro-
Acetic acid, fluoro-, sodium salt
1-Acetyl-2-thiourea
Acrolein
Aldicarb
Aldrin
Allyl alcohol
Aluminum phosphide
5-(Aminomethyl)-3-isoxazolol
4-Aminopyridine
Ammonium picrate
Ammonium vanadate
Argentate(1-), bis(cyano-C)-, potassium
Arsenic acid
Arsenic oxide
Arsenic pentoxide
Arsenic trioxide
Arsine, diethyl-
Arsonous dichloride, phenyl-
Aziridine
Aziridine, 2-methyl-
Barium cyanide
Benzenamine, 4-chloro-
Benzenamine, 4-nitro-
Benzene, (chloromethyl)-
1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-
Benzeneethanamine, alpha,alpha-dimethyl-
Benzenethiol
2H-1-Benzopyran-2-one, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-, & salts, when present at concentrations greater than 0.3%
Benzyl chloride
Beryllium
Bromoacetone
Brucine
2-Butanone, 3,3-dimethyl-1-(methylthio)-, O-[methylamino)carbonyl] oxime
Calcium cyanide
Carbon disulfide
Carbonic dichloride
Chloroacetaldehyde
p-Chloroaniline
1-(o-Chlorophenyl)thiourea

3-Chloropropionitrile
Copper cyanide
Cyanides (soluble cyanide salts), not otherwise specified
Cyanogen
Cyanogen chloride
2-Cyclohexyl-4,6-dinitrophenol
Dichloromethyl ether
Dichlorophenylarsine
Dieldrin
Diethylarsine
Diethyl-p-nitrophenyl phosphate
O,O-Diethyl O-pyrazinyl phosphorothioate
Diisopropylfluorophosphate
1,4,5,8-Dimethanonaphthalene,1,2,3,4,10,10-hexa-chloro-1,4,4a,5,8,8a,-hexahydro (1alpha,4alpha,4abeta,5alpha,8alpha,8abeta)-
1,4,5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexa-chloro-1,4,4a,5,8,8a-hexahydro-, (1alpha,4alpha,4abeta,5beta,8beta,8abeta)-
2,7:3,6-Dimethanonaphth[2,3-b]oxirene,3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-, (1aalpha,2beta,2aalpha,3beta,6beta,6aalpha,7beta, 7aalpha)-
2,7:3,6-Dimethanonaphth [2,3-b]oxirene,3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro (1aalpha,2beta,2abeta,3alpha,6alpha,6abeta,7beta,7aalpha)-, &metabolites
Dimethoate
alpha,alpha-Dimethylphenethylamine
4,6-Dinitro-o-cresol, & salts
2,4-Dinitrophenol
Dinoseb
Diphosphoramide, octamethyl-
Diphosphoric acid, tetraethyl ester
Disulfoton
Dithiobiuret
Endosulfan
Endothall
Endrin
Endrin, & metabolites
Epinephrine
Ethanedinitrile
Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-, methyl ester
Ethyl cyanide
Ethyleneimine
Famphur
Fluorine
Fluoroacetamide
Fluoroacetic acid, sodium salt
Fulminic acid, mercury(2+) salt
Heptachlor
Hexaethyl tetraphosphate

Hydrazinecarbothioamide
Hydrazine, methyl-
Hydrocyanic acid
Hydrogen cyanide
Hydrogen phosphide
Isodrin
3(2H)-Isoxazolone, 5-(aminomethyl)-
Mercury, (acetato-O)phenyl-
Mercury fulminate
Methanamine, N-methyl-N-nitroso-
Methane, isocyanato-
Methane, oxybis[chloro-
Methane, tetranitro-
Methanethiol, trichloro-
6,9-Methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-, 3-oxide
4,7-Methano-1H-indene, 1,4,5,6,7,8,8-heptachloro-3a,4,7,7atetrahydro-
Methomyl
Methyl hydrazine
Methyl isocyanate
2-Methyllactonitrile
Methyl parathion
alpha-Naphthylthiourea
Nickel carbonyl
Nickel cyanide
Nicotine, & salts
Nitric oxide
p-Nitroaniline
Nitrogen dioxide
Nitrogen oxide
Nitroglycerine (R)
N-Nitrosodimethylamine
N-Nitrosomethylvinylamine
Octamethylpyrophosphoramide
Osmium oxide, (T-4)-
Osmium tetroxide
7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid
Parathion
Phenol, 2-cyclohexyl-4,6-dinitro-
Phenol, 2,4-dinitro-
Phenol, 2-methyl-4,6-dinitro-, & salts
Phenol, 2-(1-methylpropyl)-4,6-dinitro-
Phenol, 2,4,6-trinitro-, ammonium salt
Phenylmercury acetate
Phenylthiourea
Phorate

Phosgene
Phosphine
Phosphoric acid, diethyl 4-nitrophenyl ester
Phosphorodithioic acid, O,O-diethyl S-[2-(ethylthio)ethyl] ester
Phosphorodithioic acid, O,O-diethyl S-[(ethylthio)methyl] ester
Phosphorodithioic acid, O,O-dimethyl S-[2-(methylamino) -2-oxoethyl] ester
Phosphorofluoridic acid, bis(1-methylethyl) ester
Phosphorothioic acid, O,O-diethyl O-(4-nitrophenyl) ester
Phosphorothioic acid, O,O-diethyl O-pyrazinyl ester
Phosphorothioic acid, O-[4-[(dimethylamino)sulfonyl]phenyl] O,O-dimethyl ester
Phosphorothioic acid, O,O,-dimethyl O-(4-nitrophenyl) ester
Plumbane, tetraethyl-
Potassium cyanide
Potassium silver cyanide
Propanal, 2-methyl-2-(methylthio)-, O-[(methylamino)carbonyl]oxime
Propanenitrile
Propanenitrile, 3-chloro-
Propanenitrile, 2-hydroxy-2-methyl-
1,2,3-Propanetriol, trinitrate
2-Propanone, 1-bromo-
Propargyl alcohol
2-Propenal
2-Propen-1-ol
1,2-Propylenimine
2-Propyn-1-ol
4-Pyridinamine
Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-, & salts
Selenious acid, dithallium(1+) salt
Selenourea
Silver cyanide
Sodium azide
Sodium cyanide
Strychnidin-10-one, & salts
Strychnidin-10-one, 2,3-dimethoxy-
Strychnine, & salts
Sulfuric acid, dithallium(1+) salt
Tetraethyldithiopyrophosphate
Tetraethyl lead
Tetraethyl pyrophosphate
Tetranitromethane
Tetraphosphoric acid, hexaethyl ester
Thallic oxide
Thallium oxide
Thallium(I) selenite
Thallium(I) sulfate
Thiodiphosphoric acid, tetraethyl ester

Thiofanox
Thioimidodicarbonic diamide
Thiophenol
Thiosemicarbazide
Thiourea, (2-chlorophenyl)-
Thiourea, 1-naphthalenyl-
Thiourea, phenyl-
Toxaphene
Trichloromethanethiol
Vanadic acid, ammonium salt
Vanadium oxide
Vanadium pentoxide
Vinylamine, N-methyl-N-nitroso-
Warfarin, & salts, when present at concentrations greater than 0.3%
Zinc cyanide
Zinc phosphide, when present at concentrations greater than 10%