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A. Hazardous Material

1. Hazardous Material Information

Development, Preparedness, and Response Rules

(Act 435 of the 1985 Louisiana Legislative Regular Session)

The purpose of the Act is to insure that the hazards of all chemicals produced, imported, consumed, applied, transported, stored, or emitted in Louisiana are communicated to appropriate emergency response organizations, local information repositories, and to the general public upon request.

EHS's role in responding to the requirements of this Act is covered in Section VII of the manual.

2. Hazard Communication Program

a. General Program Information

The purpose of this program is to make employees aware of the hazards of chemicals and provide them information on how to protect themselves. The LSU written Hazard Communication Plan can be found in the Appendix. This plan has been developed based on the OSHA Hazard Communication Standard and addresses hazardous material identification, use of safety data sheets, employee training, and labeling.

b. Responsibilities

i. Deans, Directors, Department Chairs, Principal Investigators, Managers and Supervisors shall:

- 1) Comply with the specific requirements of the program.
- 2) Maintain a current list of chemicals in the work place. Update the list on an ongoing basis via the Internet.
- 3) Ensure that minimum amounts of chemicals are maintained in the workplace.
- 4) Ensure that SDSs are readily available to employees.
- 5) Ensure that necessary physical or toxic warning signs are posted in those areas where special notices are required.
- 6) Ensure that each work area requiring specific personal protective equipment is posted with appropriate warning signs. Department Heads/Supervisors shall make appropriate personal protective equipment available as needed.
- 7) Inform any contractor working in their area of chemicals to which their employees are exposed. Contractors must reciprocate on chemicals used in their work. SDS information shall be exchanged.
- 8) Train their employees regarding the chemicals in the workplace, the location and operation of controls, procedures used to protect themselves and other workers, emergency plans and location of SDS or information related to chemicals in the workplace. (Note: Much of the above can be handled in safety meetings.)

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- ii. The Environmental Health and Safety (EHS) Office is responsible for:
 - 1) Establishment of an SDS library/access system via the Internet.
 - 2) Assisting departments in determining proper use, storage and labeling of chemicals.
 - 3) Assisting departments with employee training. This shall be coordinated through the Safety and Environmental Training Officer.
 - 4) Assessing chemical inventories provided by LSU departments and ensure that SDSs are available via the Internet.

- iii. University Stores shall:
 - 1) Ensure that each container of a chemical received is properly labeled.
 - 2) Ensure that each container of a chemical shipped to departments is properly labeled.
 - 3) Ensure employees are properly trained in handling chemicals and in spill response.

- iv. Employees shall:
 - 1) Learn about the chemical and physical hazards of chemicals in their workplace and how to protect themselves.
 - 2) Comply with the chemical safety requirements of LSU's hazard communication program and the SDS sheets for a particular chemical.
 - 3) Immediately report spills or suspected spills of chemicals.
 - 4) Report any problems with storage or use of chemicals.
 - 5) Use only those chemicals for which they have received training.
 - 6) Use chemicals only for the tasks designated and covered in standard operating procedures, and protect other employees from these hazards.
 - 7) Inform their supervisors of changes in operations that could affect the safety and health of the job site or work area.
 - 8) Use personal protective equipment as specified by the SDS.

- v. Contractors' responsibilities:
 - 1) Comply with all aspects of LSU's Hazard Communication Program and OSHA's Hazard Communication Standard, 29 CFR 1910.1200.
 - 2) Ensure Contractor employees are properly trained.
 - 3) Monitor and ensure proper storage and use of chemicals by Contractor employees.
 - 4) Properly dispose of chemicals and hazardous waste.

c. Training Program

Department Heads/Supervisors shall provide training to affected employees annually, or when changes in operations warrant. Environmental Health and Safety (EHS) can assist in this effort. The training shall include the following points:

- i. Physical and health hazards of the chemicals or materials in the workplace and all information on the SDS that may affect employees.
- ii. Methods that may be used to detect the presence of a chemical or material by visual appearance, odor, irritation (skin, headaches, coughing).
- iii. Measures used to protect the employee (engineering design, barriers, ventilation, operating procedures, special training, etc.).
- iv. Measures employees can take to protect themselves from exposure (work practices, respiratory equipment, eye protection, other personal protective equipment, special training, etc.).
- v. Details of the Hazard Communication Program.

An outline of a hazard communication training program is contained in the appendix.

3. Hazardous Materials Definitions

a. Chemical

“Chemical” means any element, chemical compound or mixture of elements and/or compounds.

b. Combustible Liquid

“Combustible liquid” means any liquid having a flashpoint at or above 100 deg. F (37.8 deg. C), but below 200 deg. F (93.3 deg. C), except any mixture having components with flashpoints of 200 deg. F (93.3 deg. C), or higher, the total volume of which make up 99 percent or more of the total volume of the mixture.

c. Compressed Gases

- i. A gas or mixture of gases having, in a container, an absolute pressure exceeding 40 psi at 70 deg. F (21.1 deg. C); or
- ii. A gas or mixture of gases having, in a container, an absolute pressure exceeding 104 psi at 130 deg. F (54.4 deg. C) regardless of the pressure at 70 deg. F (21.1 deg. C); or
- iii. A liquid having a vapor pressure exceeding 40 psi at 100 deg. F (37.8 deg. C) as determined by ASTM D-323-72.

d. Container

“Container” means any bag, barrel, bottle, box, can, cylinder, drum, reaction vessel, storage tank, or the like that contains a hazardous chemical. For purposes of this section, pipes or piping systems, and engines, fuel tanks, or other operating systems in a vehicle, are not considered to be containers.

e. Explosive

“Explosive” means a chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.

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f. Exposure or Exposed

“Exposure or exposed” means that an employee is subjected in the course of employment to a chemical that is a physical or health hazard, and includes potential (e.g. accidental or possible) exposure. “Subjected” in terms of health hazards includes any route of entry (e.g. inhalation, ingestion, skin contact or absorption.)

g. Flammable

“Flammable” means a chemical that falls into one of the following categories:

- i. “Aerosol, flammable” means an aerosol that, when tested by the an approved method described yields a flame projection exceeding 18 inches at full valve opening, or a flashback (a flame extending back to the valve) at any degree of valve opening;
- ii. “Gas, flammable” means:
 - 1) A gas that, at ambient temperature and pressure, forms a flammable mixture with air at a concentration of thirteen (13) percent by volume or less; or
 - 2) A gas that, at ambient temperature and pressure, forms a range of flammable mixtures with air wider than twelve (12) percent by volume, regardless of the lower limit;
- iii. “Liquid, flammable” means any liquid having a flashpoint below 100 deg. F (37.8 deg. C), except any mixture having components with flashpoints of 100 deg. F (37.8 deg. C) or higher, the total of which make up 99 percent or more of the total volume of the mixture.
- iv. “Solid, flammable” means a solid, other than a blasting agent or explosive, that is liable to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a serious hazard. A chemical shall be considered to be a flammable solid if, when tested by an approved method, it ignites and burns with a self-sustained flame at a rate greater than one-tenth of an inch per second along its major axis.

h. Flashpoint

“Flashpoint” means the minimum temperature at which a liquid gives off a vapor in sufficient concentration to ignite when tested as follows:

- i. Tagliabue Closed Tester (See American National Standard Method of Test for Flash Point by Tag Closed Tester, Z11.24-1979 (ASTM D 56-79)) for liquids with a viscosity of less than 45 Saybolt Universal Seconds (SUS) at 100 deg. F (37.8 deg. C), that do not contain suspended solids and do not have a tendency to form a surface film under test; or
- ii. Pensky-Martens Closed Tester (see American National Standard Method of Test for Flash Point by Pensky-Martens Closed Tester, Z11.7-1979 (ASTM D 93-79)) for liquids with a viscosity equal to or greater than 45 SUS at 100 deg. F (37.8 deg. C), or that contain suspended solids, or that have a tendency to form a surface film under test; or

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iii. Setaflash Closed Tester (see American National Standard Method of Test for Flash Point by Setaflash Closed Tester (ASTM D 3278-78)).

Organic peroxides, which undergo autoaccelerating thermal decomposition, are excluded from any of the flashpoint determination methods specified above.

i. Hazard Warning

“Hazard warning” means any words, pictures, symbols, or combination thereof appearing on a label or other appropriate form of warning which convey the specific physical and health hazard(s), including target organ effects, of the chemical(s) in the container(s). (See the definitions for “physical hazard” and “health hazard” to determine the hazards which must be covered.)

j. Hazardous Chemical

“Hazardous chemical” means any chemical which is a physical hazard or a health hazard.

k. Health Hazard

“Health hazard” means a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees. The term “health hazard” includes chemicals which are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents which act on the hematopoietic system, and agents which damage the lungs, skin, eyes, or mucous membranes. Appendix A provides further definitions and explanations of the scope of health hazards covered by this section, and Appendix B describes the criteria to be used to determine whether or not a chemical is to be considered hazardous for purposes of this standard.

l. The NFPA Hazard Identification System (Diamond)

Red

Fire

Blue Yellow

Health Reactivity

White

Colorless

Numerical rating from 0-4 with the hazards becoming more extreme as they are upscaled.

Health–Blue

4 A few whiffs of the gas or vapor could cause death; liquid penetration could be fatal.

3 Material is extremely dangerous, but area may be entered if fully protected.

2 Material hazardous to health, but area may be entered freely if SCBA is used.

1 Material is only slightly hazardous to health.

0 Material offers no health hazard.

Fire–Red

4 Flash point below 73 deg F.

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- 3 Flash point below 100 deg F.
- 2 Flash point between 100 and 200 deg F.
- 1 Flash point above 200 deg F.

0 Materials that will not burn.

Reactivity–Yellow

4 May detonate

3 Shock and heat may detonate

2 Violent chemical change

1 Unstable if heated

0 Stable.

Colorless

Special reactive materials such as:

Oxidizers: OX

Corrosive: CORR

Radiation:

Water Reactive: -W-

m. Organic Peroxide

“Organic peroxide” means an organic compound that contains the bivalent -O-O- structure and which may be considered to be a structural derivative of hydrogen peroxide where one or both of the hydrogen atoms has been replaced by an organic radical.

n. Oxidizer

“Oxidizer” means a chemical other than a blasting agent or explosive as defined in 1910.109(a), that initiates or promotes combustion in other materials, thereby causing fire either of itself or through the release of oxygen or other gases.

o. Physical Hazard

“Physical hazard” means a chemical for which there is scientifically valid evidence that it is a combustible liquid, a compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable (reactive) or water-reactive.

p. Pyrophoric

“Pyrophoric” means a chemical that will ignite spontaneously in air at a temperature of 130 deg. F (54.4 deg. C) or below.

q. Reactive Chemicals

“Unstable (reactive)” means a chemical which in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shocks, pressure or temperature. “Water-reactive” means a chemical that reacts with water to release a gas that is either flammable or presents a health hazard.

4. Chemical Handling and Storage

a. General Considerations

- i. Avoid storing equipment and material on top of cabinets.

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- ii. Do not store materials on top of high cabinets where they will be difficult to reach.
- iii. Keep exits, passageways, areas under tables or benches, and emergency equipment areas free of stored equipment and materials.
- iv. Label all chemical containers appropriately.
- v. Place the users name and the date received on all purchased materials in order to facilitate inventory control of the materials.
- vi. Provide a definite storage place for each chemical and return the chemical to that location after each use.
- vii. Avoid storing chemicals on bench tops, except of those chemicals being used currently.
- viii. Avoid storing chemicals in laboratory hoods, except for those being used currently.
- ix. Store volatile toxins and odoriferous chemicals in a ventilated cabinet. Check with the institution's environmental health and safety officer.
- x. Provide ventilated storage near laboratory hoods.
- xi. If a chemical does not require a ventilated cabinet, store it inside a closable cabinet or on a shelf that has a lip to prevent containers from sliding off in the event of a fire, serious accident, or earthquake.
- xii. Do not expose stored chemicals to heat or direct sunlight.
- xiii. Observe all precautions regarding the storage of incompatible chemicals.
- xiv. Separate chemicals into compatible groups and store alphabetically within compatible groups. See below for one suggested method for arranging chemicals in this way.

Related and Compatible Storage Groups:

Inorganic Family

Metals, hydrides

Halides, sulfates, sulfites, thiosulfates, phosphates, halogens

Amides, nitrates (except ammonium nitrate), nitrites, azides

Hydroxides, oxides, silicates, carbonates, carbon

Sulfides, selenides, phosphides, carbides, nitrides

Chlorates, perchlorates, perchloric acid, chlorites, hypochlorites, peroxides,
hydrogen peroxide

Arsenates, cyanides, cyanates

Borates, chromates, manganates, permanganates,

Nitric acid, other inorganic acids

Sulfur, phosphorus, arsenic, phosphorus pentoxide

Organic Family

Acids, anhydrides, peracids

Alcohols, glycols, amines, amides, imines, imides

Hydrocarbons, esters, aldehydes

Ethers, ketones, ketenes, halogenated hydrocarbons, ethylene oxide

Epoxy compounds, isocyanates

Peroxides, hydroperoxides, azides

Sulfides, polysulfides, sulfoxides, nitrites

Phenols, cresols

xv. Store flammable liquids in approved flammable liquid storage cabinets.

b. Containers and Equipment

- i. Use corrosion-resistant storage trays or secondary containers to retain materials if the primary container breaks or leaks.
- ii. Provide vented cabinets beneath laboratory hoods for storing hazardous materials. (This encourages the use of the hoods for transferring such materials.)
- iii. Use chemical storage refrigerators only for storing chemicals.
- iv. Label these refrigerators with the following signage: No Food-Chemical Storage
- v. Seal containers to minimize escape of corrosive, flammable, or toxic vapors.
- vi. Label all materials in the refrigerator with contents, owner, date of acquisition or preparation, and nature of any potential hazard.
- vii. Do not store flammable liquids in a refrigerator unless it is approved for such storage. Such refrigerators are designed not to spark inside the refrigerator. If refrigerated storage is needed inside a flammable-storage room, it is advisable to choose an explosion-proof refrigerator.

c. Storing Highly Reactive Substances

- i. Consider the storage requirements of each highly reactive chemical prior to bringing it into the laboratory.
- ii. Consult the SDSs or other literature in making decisions about storage of highly reactive chemicals.
- iii. Bring into the laboratory only the quantities of material you will need for your immediate purposes (less than 3- to 6-month supply, the length depending on the nature and sensitivity of the materials).
- iv. Label, date, and inventory all highly reactive materials as soon as received. Make sure the label states, **Danger! Highly Reactive Material!**
- v. Do not open a container of highly reactive material that is past its expiration date. Call your institution's hazardous waste coordinator for special instructions.
- vi. Do not open a liquid organic peroxide or peroxide former if crystals or a precipitate are present. Call your institution's hazardous waste coordinator for special instructions.
- vii. Dispose of (or recycle) highly reactive material prior to expiration date.
- viii. Segregate the following materials:
 - 1) oxidizing agents from reducing agents and combustibles
 - 2) powerful reducing agents from readily reducible substrates
 - 3) pyrophoric compounds from flammables, and
 - 4) perchloric acid from reducing agents.
- ix. Store highly reactive liquids in trays large enough to hold the contents of the bottles.

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- x. Store perchloric acid bottles in glass or ceramic trays.
 - xi. Store peroxidizable materials away from heat and light.
 - xii. Store materials that react vigorously with water away from possible contact with water.
 - xiii. Store thermally unstable materials in a refrigerator. Use a refrigerator with these safety features:
 - 1) all spark-producing controls on the outside,
 - 2) a magnetic locked door, and
 - 3) an alarm to warn when the temperature is too high.
 - xiv. Store liquid organic peroxides at the lowest possible temperature consistent with the solubility or freezing point. Liquid peroxides are particularly sensitive during phase changes.
 - xv. Inspect and test-peroxide-forming chemicals periodically (these should be labeled with an acquisition or expiration date) and discard containers that have exceeded their safe storage lifetime.
 - xvi. Store particularly sensitive materials or larger amounts of explosive materials in explosive relief boxes.
 - xvii. Restrict access to the storage facility.
 - xviii. Assign responsibility for the storage facility to one primary person and a backup person. Review this responsibility at least yearly.
- d. Storing Toxic Substances
- i. Store chemicals known to be highly toxic (including carcinogens) in ventilated storage in unbreakable, chemically resistant secondary containers.
 - ii. Keep quantities at a minimum working level.
 - iii. Label storage areas with appropriate warning signs, such as:
Caution! Reproductive Toxin Storage or
Caution! Cancer-Suspect Agent Storage

5. Biohazard Control

This section presents certain safety requirements for handling specific hazardous microorganisms. These requirements are based on present knowledge and shall change as research continues and new standards are developed. All activities within labs where biohazards exist must operate under the CDC/NIH guidelines for biosafety. These guidelines are on the EHS web page. The operational requirements serve, in part, to indicate what facilities and resources should be made available to minimize hazards in work with specific microorganisms.

a. General Guidelines.

i. Authorized Personnel

Only authorized employees, students, and visitors shall be allowed to enter infectious disease laboratories or utility rooms.

ii. Autoclaving

- 1) All infectious or toxic materials, equipment, or apparatus shall be autoclaved or otherwise disinfected before being washed or disposed of. Each individual working with infectious material shall be responsible for its disinfection before disposal.
- 2) Infectious or toxic materials shall not be placed in autoclaves overnight in anticipation of autoclaving the next day.
- 3) Autoclaves shall be checked for operating efficiency if spore formers are used.

iii. Building Vacuum Line

When the building vacuum line is used, suitable traps or filters shall be interposed to insure that pathogens do not enter the fixed system.

iv. Centrifuging, Sonication, Separation–Aerosol Precautions

- 1) Before centrifuging, inspect tubes for cracks; inspect the inside of the trunnion cup for rough walls caused by erosion of adhering matter; and carefully remove bits of glass from the rubber cushion. A germicidal solution added between the tube and trunnion cup not only disinfects the outer surface of both of these but also provides an excellent cushion against shocks that might otherwise break the tube.
- 2) Avoid decanting centrifuge tubes. If you must do so, wipe off the outer rim with a disinfectant afterwards; otherwise, the infectious fluid will spin off as an aerosol. Avoid filling the tube to the point that the rim becomes wet with culture.
- 3) A ventilated and filtered safety centrifuge cabinet is recommended to house and safeguard all centrifuging of infectious substances. Use a safety centrifuge cup. Centrifuging shall always be done in closed containers and, whenever possible, in closed centrifuge heads. When centrifuging is done in a ventilated glove box, the glove panel shall be in place with the glove ports covered. A centrifuge in operation creates reverse air currents that may cause escape of agent from an open cabinet.
- 4) An aerosol containment hood or enclosure shall be provided for sonicators, cream separators, and similar aerosol producing apparatus.

v. Containers–Protective

- 1) Insure that all virulent fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily-handled, non-breakable, leak-proof containers that are large enough to contain all fluid or powder in case of leakage or breakage of the glass vessel.
- 2) All inoculated Petri plates or other inoculated solid media shall be transported and incubated in leak-proof pans or other leak-proof containers.

vi. Emergencies

- 1) No person shall work alone on an extremely hazardous operation. Use the “buddy system.”

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- 2) Prepared solutions of suitable disinfectants, along with instructions for use, should be maintained in each laboratory in a conspicuous location. The location should be labeled “Disinfectants for Emergency Use.”
 - 3) An Emergency Notification Sign, available from the Office of Environmental Health and Safety, shall be posted by the responsible researcher on the exterior door of each lab giving instructions to follow in the event of an emergency such as a fire or spill. Instructions shall emphasize precautionary measures. Biohazard signs must be posted on lab doors.
- vii. Floors and Other Surfaces
- 1) Floors, laboratory benches, and other surfaces in the buildings in which infectious substances are handled shall be disinfected with a suitable germicide as often as deemed necessary by the supervisors or as directed by CDC/NIH guidelines. After completion of operations involving planting, pipetting, centrifuging, lyophilizing, and similar procedures with infectious agents, the surroundings shall be disinfected.
 - 2) Floor drains throughout the building shall be flooded with water, glycol, or a safe disinfectant at least once a week in order to fill traps and prevent backflow of sewer gases.
 - 3) Elimination of sweeping through use of vacuum cleaners utilizing absolute filters or through wet mopping only is recommended. If floors are swept, use push brooms only. The use of a floor sweeping compound is recommended because of its effectiveness in lowering the number of airborne organisms. Water used to mop floors shall contain suitable disinfectants. Custodial services in Biosafety Level 3 or 4 labs shall be performed by laboratory personnel only—not by Facility Services or University contractors.
- viii. Hygiene
- 1) Develop the habit of keeping your hands away from your mouth, nose, eyes, and face. This may prevent self-inoculation.
 - 2) Deepfreeze and dry ice chests and refrigerators shall be checked and cleaned out periodically to remove broken ampules, tubes, etc., containing infectious material. A tray can be used to line the bottom of the refrigerator or freezer to catch and retain broken containers.
 - 3) Library books and journals shall not be taken into rooms where work with infectious agents is in progress.
 - 4) An effort shall be made to keep all other surplus materials and equipment out of these rooms.
 - 5) According to the level of risk, the wearing of laboratory or protective clothing may be required for persons entering infectious disease laboratories. Likewise, showers with a germicidal soap may be required before exit.
 - 6) Contaminated laboratory clothing shall not be worn in clean areas or outside the building.

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- ix. Labeling
All laboratory rooms containing infectious substances shall designate separate areas or shelters labeled: “Infectious–To Be Autoclaved,” or “Not Infectious–To Be Cleaned.” All infectious disease work areas including cabinetry shall be prominently marked with the Biohazard Warning Control symbol. Cultures shall be labeled with the name of the agent, researchers’ names, and date.
- x. Membrane Filters
Care shall be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as noninfectious until culture or other tests have proven their sterility.
- xi. Personal Protective Equipment
 - 1) A respirator or gas mask shall be worn when changing a glove or gloves attached to a safety cabinet if an infectious aerosol may possibly be present in the cabinet. See “Personal Protective Equipment.”
 - 2) Diagnostic serum specimens carrying a risk of serum hepatitis shall be handled with rubber gloves.
- xii. Pest Control
Pest and rodent control must be maintained. (See CDC/NIH Guidelines) All laboratories shall be sprayed with insecticides as often as necessary to control flies and other insects. Consult Physical Plant pesticide control personnel for spraying. Advise them of hazards in the laboratory before work begins and exercise adequate control of entry and activity as permitted under the lab classification.
- xiii. Pipettes
 - 1) No infectious materials shall be pipetted by mouth or blown out of a pipette. Do not use a pipette for mixing or for bubbling air through an infectious mixture. Hand pipetting devices shall be used to pipette all microorganisms, tissue, cell cultures, caustic or corrosive chemicals, poisons, organic solvents, radioactive materials, mutagens, carcinogens, or teratogens.
 - 2) Contaminated pipettes shall be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion. They shall not be placed vertically in a cylinder. The pan and pipette shall be autoclaved as a unit and replaced by a clean pan with fresh disinfectant.
- xiv. Syringes
 - 1) Only syringes of the Luer-Lok type shall be used with infectious materials.
 - 2) Use an alcohol-soaked pledget around the stopper and needle when removing a syringe and needle from a rubber-stoppered vaccine bottle.
 - 3) Expel excess fluid and bubbles from a syringe vertically into a cotton pledget soaked with disinfectant or into a small bottle or cotton.
 - 4) Syringes and needles shall be autoclaved and destroyed before disposal into special waste containers (not into the “trash”). Blunt needles (cannulas) shall be used wherever sharp needles are not required.

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- xv. Shakers
 - 1) A safety box or safety shaker tray shall be used to house or safeguard all containers of infectious substances on shaking machines.
 - 2) Broth cultures shall be taken in a manner that avoids wetting the plug or cap.
 - xvi. Smoking, Eating, and Drinking
 - 1) Food, candy, gum, or beverages for human consumption shall not be taken into infectious disease or recombinant DNA research laboratories. Smoking shall not be permitted in any area in which work with infectious or toxic substances is performed. Employees who have been working with infectious materials shall wash and disinfect their hands thoroughly before smoking, eating, or drinking. See “Consumption of Food and Beverages.”
 - 2) Industrial water from lab sinks shall not be used for human consumption.
 - xvii. Storage

At the close of each workday, to minimize hazard to fireman, maintenance personnel, or emergency crews, all infectious or toxic material shall be:

 - 1) placed in the refrigerator,
 - 2) placed in the incubator, or
 - 3) autoclaved or otherwise disinfected before the building is closed.
 - xviii. Wastes
 - 1) No infectious substances shall be allowed to enter a building drainage or refuse disposal system without proper sterilization.
 - 2) Mechanical garbage disposal units shall not be installed for use in disposing of contaminated wastes. The units release considerable amounts of aerosol.
 - xix. Water Baths and Warburg Baths

Water baths and warburg baths used to inactivate, incubate, or test infectious substances shall contain a suitable disinfectant. For cold water baths, 70% propylene glycol is recommended.
- b. Sterilization and Disinfection Methods
- General criteria for sterilization of typical materials are presented below. Supervisors are encouraged to review the type of materials being handled and to establish standard conditions for sterilization. Treatment conditions to achieve sterility will vary in relation to the volume of material treated, its contamination level, the moisture content, and other factors.
- i. Steam Autoclave
 - 1) Laundry–250F (121C) for 30 minutes with 15 minutes pre-vacuum of 27" Hg.
 - 2) Trash–250F (121C) for 1 hour with 15 minutes pre- vacuum of 27" Hg.
 - 3) Glassware–250F (121C) for 1 hour with 15 minutes pre-vacuum of 27" Hg for filled NTH Glassware can.
 - 4) Liquids–250F (121C) for 1 hour for each gallon.
 - 5) Animals–250F (121C) for 8 hours with 15 minutes pre-vacuum of 27" Hg.
 - 6) Bedding–250F (121C) for 8 hours with 15 minutes pre-vacuum of 27" Hg.

ii. Gas Sterilants

- 1) Ethylene Oxide Gas—Sixteen hours exposure to a concentration of 750 mg/liter (approximately 5%) at 30 to 60% relative humidity and at ambient temperatures (> 70F).
- 2) Paraformaldehyde—16 hours exposure to a concentration of 1.0 mg/liter at 40 to 60% relative humidity at ambient temperatures (> 70F).

iii. Disinfectants

- 1) Mercurials are not recommended for general use because they have poor activity against vegetative bacteria and are useless as sporicides. Although the mercurials exhibit good activity against viruses (1:500 to 1:1000 concentration), they are toxic and not recommended.
- 2) Quaternary Ammonium Compounds are acceptable as general-use disinfectants to control vegetative bacteria and non-lipid-containing viruses. However, they are not active against bacterial spores at the usual-use concentrations (1.750).
- 3) Phenolic Compounds are recommended for killing vegetative bacteria, including Mycobacterium tuberculosis, fungi, and lipid-containing viruses.
- 4) Chlorine Compounds are recommended for certain disinfecting procedures, provided the available chlorine needed is considered. Low concentrations of available chlorine (50 to 500 ppm) are active against vegetative bacteria and most viruses. For bacterial spores, concentrations of approximately 2500 ppm are needed. The corrosive nature of these compounds, their decay rates, and lack of residuals are such that they are recommended only in special situations.
- 5) Iodophors show poor activity against bacterial spores, but they are recommended for general use (75 to 150 ppm). They are effective against vegetative bacteria and viruses. Their advantages are:
 - a) Iodophors possess a wide spectrum of antimicrobial and antiviral activity.
 - b) Iodophors have a built-in indicator. If the solution is brown or yellow, it is still active.
 - c) Iodophors are relatively harmless to man.
 - d) Iodophors can be readily inactivated and iodophor stains can be readily removed with solutions of Na₂S₂O₃ (Sodium Thiosulfate).
- 6) Alcohols, in concentrations of 70 to 95 percent, are good general-use disinfectants, but they exhibit no activity against bacterial spores.
- 7) Formaldehyde Solutions, in concentrations of 8 percent, exhibit good activity against vegetative bacteria, spores, and viruses.
- 8) Activated Glutaraldehyde, in two percent solutions, exhibit good activity against vegetative bacteria, spores, and viruses. Its use, however, shall be limited and controlled because of its toxic properties and the damage to the eyes.
- 9) Formaldehyde-Alcohol, in solutions of 8 percent in 70 percent alcohol are considered very good for disinfection purposes because of their effectiveness against vegetative bacteria, spores, and viruses. For many applications, this the disinfectant of choice.

c. Factors Influencing Biological Safety Equipment Selection

- i. Proposed Activity. Procedures which may cause aerosols to be of particular concern.
- ii. Risk of the Infective Agent. All known characteristics of the agent shall be evaluated, i.e., potential for infection, history of known laboratory-acquired human infections, concentration of the viable agent to be used, classification of the etiologic agent on the basis of hazard, etc.
- iii. Control Objectives. The control protection desired shall be determined from the proposed activity and the specific agent.
 - 1) Product protection only
 - 2) Personnel protection only
 - 3) Personnel and product protection

d. Biosafety Ventilation Equipment

See Appendix

e. Animals

Plans for a biohazard program involving animals shall be submitted to the appropriate department head and reviewed and approved as necessary by the Animal Committee. All animals, equipment, and the animal room itself shall be treated as contaminated. Each animal shall be identified to indicate inoculation with infectious substances.

i. Animal Cages

Cages used for infected animals shall be cared for in the following manner:

- 1) Careful handling procedures shall be employed to minimize the dissemination of dust from cage refuse and animals.
- 2) Cages shall be sterilized by autoclaving. Refuse bowls and watering devices shall remain in the cage during sterilization.
- 3) All watering devices shall be of a non-drip type.
- 4) Each cage shall be examined each morning and at each feeding time so that dead animals can be removed.
- 5) Animals in cages with shavings shall be transferred to clean cages as often as necessary. If cages have false screen platforms, the catch pan shall be replaced before it becomes full.
- 6) The names of the investigators, a description of infectious agent, the date and method of administration, and an emergency telephone number shall be placed on each cage.
- 7) If properly maintained, ultraviolet lamps and reflectors can prevent the airborne spread of infections between cages. Depending upon the location of the ultraviolet lamps, it may be necessary to shield the lamp to protect animals and personnel from eye damage. Protective goggles may be necessary. High efficiency spun-glass filter materials used on the sides or top of a small animal cage will also prevent cross-infection. Consult the Office of Environmental Health and Safety.

- 8) Several types of ventilated cages are available and useful where airborne organisms are under investigation. Ventilated lids can be made to fit ordinary animal cages by use of air-tight gaskets around the rim of the lid, which are connected to a central exhaust system through an absolute filter. Horsefall type cubicles of flexible film isolators may also be used.
- ii. Animal Rooms
- 1) Doors to animal rooms shall be kept closed at all times except for necessary entrance and exit. The doors shall be marked by a conspicuous sign, Hazardous Biological Materials. The conventional biohazard symbol shall be used.
 - 2) Unauthorized persons shall not be permitted entry to animal rooms.
 - 3) A container of suitable disinfectant shall be kept in each animal room for disinfecting gloves and boots and for general decontamination. Floors, walls, and cage racks shall be mop-washed with a suitable disinfectant frequently.
 - 4) Floor drains in animal rooms shall be flooded with water, glycol, or a suitable disinfectant periodically to prevent backing up of sewer gases.
 - 5) Shavings or other refuse on floors shall not be washed down the floor drains.
 - 6) Animal rooms shall have a licensed pest control service.
 - 7) Special care shall be taken to prevent live animals, especially mice, from finding their way in disposable trash.
 - 8) Animal rooms in infectious disease units shall be ventilated under negative pressure with respect to corridors or adjoining non-infectious areas. Ten to fifteen changes of air per hour generally are sufficient, depending upon the species of animal. There shall be no recirculating of room air in infectious areas.
 - 9) Use disinfectant vaporizers to decontaminate an animal room after experiments. No personnel or animals shall be in the room during this process.
- iii. Handling Infected Animals
- 1) Special attention shall be given to the humane treatment of all laboratory animals in accordance with the Principles of Laboratory Animal Care as promulgated by the National Society for Medical Research and the National Institute of Health.
 - 2) Monkeys shall be tuberculin-tested and examined for herpes lesions.
 - 3) Suitable masks and eye protection shall be worn when primates are being handled.
 - 4) Persons regularly handling monkeys shall receive periodic chest X-ray examinations and other appropriate tuberculosis detection procedures.
 - 5) Heavy gloves shall be worn when feeding, watering, or removing infected animals. Under no circumstances shall the bare hands be placed in the cage to move any object.
 - 6) When animals are to be injected with pathogenic material, the animal caretaker shall wear protective gloves and the laboratory workers should wear surgeon's gloves. Every effort shall be made to restrain the animal to avoid accidents that

may result in disseminating infectious materials. Before and after injection of an animal, swab the site of the injection with a disinfectant.

- 7) Ventilated biological safety cabinets or laminar flow biological hoods are recommended for the inoculation of animals with infectious organisms and for necropsy of infected animals. They shall be equipped, as applicable, with viewing windows, glove ports, lights, ultraviolet lamps, an air exhaust absolute filter, and outlets for gas, air, water, and vacuum.
- 8) Infected animals to be transferred via public corridors shall be placed in aerosol-proof containers.

iv. Animal Bites

- 1) If the handler receives a bite or scratch, the wound (even a superficial one) shall be scrubbed for three minutes with soap and water followed by a thorough rinsing with warm water, drying with an absorbent cotton, and swabbing with 1% solution of Zephiran Chloride. The injured person shall seek medical attention promptly. If a fever develops, the handler shall report for medical attention promptly. If a fever develops, the handler shall report for medical aid and inform the doctor that he works with animals.
- 2) Report any bite wounds to employees on an Employers Report of Occupational Injury or Disease Report. Students shall report bite wounds to their instructors and to the Student Health Center. Appropriate medical care shall be sought immediately.

v. Necropsy of Infected Animals

- 1) Necropsy of infected animals shall be carried out in ventilated biological safety cabinets equipped with absolute exhaust filtration or special rooms with such ventilation control. The inside of the ventilated cabinet and other potentially contaminated surfaces shall be disinfected with a suitable germicide before and after the necropsy.
- 2) Rubber gloves shall be worn when performing necropsies. Respiratory protection shall be used to prevent the inhalation of infectious agents.
- 3) Surgeon's gowns shall be worn over laboratory clothing during necropsies.
- 4) Fur of the animal shall be wet with a suitable disinfectant.
- 5) Small animals shall be restrained and placed within a metal tray. Large animals shall be processed in an appropriate room designated for the purpose of necropsy.
- 6) Upon completion of necropsy, all potentially contaminated material shall be placed in suitable disinfectant or left in the necropsy tray. The entire tray shall be autoclaved at the conclusion of the operation.
- 7) Grossly contaminated rubber gloves shall be cleaned in disinfectant before removal from hands, preparatory to sterilization.
- 8) Dead laboratory animals shall be placed in proper leak-proof containers and thoroughly autoclaved before removal and incineration if zoonotic potential is present.

vi. Recommended Biosafety Levels for Infectious and Infected Animals

The selection of an appropriate biosafety level for work with a particular agent or animal study is dependent upon a number of factors. The most important of these include: the virulence, pathogenicity, biological stability, communicability of the agent, the nature of function of the laboratory or function, the quantity and concentration of the agent, the endemicity of the agent, and the availability of effective vaccines or therapeutic measures.

The laboratory supervisor and/or principal investigator shall seek approval of the Department Head and the Institutional Biological and Recombinant DNA Safety Committee as well as other necessary administrative directors prior to initiation of experiments dealing with bio-hazard materials. The principal criteria to be satisfied is the safety of University personnel and students. It is also necessary to comply with CDC/NIH guidelines.

If a combination of increasingly stringent primary and secondary containment procedures and facilities is used, laboratory studies and manipulations can be safely conducted on agents that are correspondingly more hazardous.

In general, the biosafety level used for activities using infectious agents or infected animals shall be commensurate with that required for the agent of highest virulence known or likely to be encountered in the course of contemplated work. For example: all diagnostic sera of human origin shall be considered potentially infectious for hepatitis and handled under conditions which reasonably preclude cutaneous, oral, and parenteral exposure to personnel. Sputa shall be considered as potentially infectious for tuberculosis and should be handled under conditions which reasonably preclude the generation of aerosols, or which contain any aerosols generated. If, in the course of diagnostic or other laboratory examinations, there is evidence that the materials being studied contain only an agent of higher or lower risk than expected, the biosafety level shall be raised or lowered accordingly.

f. Inspection of Bioscience Laboratories

i. Responsibilities.

- 1) The assignment of inspection personnel depends upon the type of inspection and area to be covered. Safety of assigned areas is inherently the responsibility of the supervisor. To detect unsafe conditions, he/she may assign inspection duties to subordinates or use available staff inspection groups. In any event, the responsibility rests with the supervisor.
- 2) Inspections of Biosafety Level 3 Recombinant DNA research labs must be performed by the Biosafety Officer. Such inspections are recommended for labs performing work with infectious agents.

ii. Procedures.

- 1) To help with the inspection process, an inspection report form has been developed that includes items construed to be important by the National

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- Institute of Health, American Chemical Society, and the National Safety Council regarding safety in the bio-science laboratory. (See Appendix.)
- 2) Once finalized, the report shall be turned over to the department head/chairperson and a copy sent to the Office of Environmental Health and Safety (EHS).
 - 3) The department head/chairperson shall review the report and respond by taking measures necessary to comply with recommendations.
 - 4) It is important that the inspected area be identified by building name, department (botany, zoology, physiology, etc.), floor on which it is located, and room number. Furthermore, the area shall be identified as used for teaching, research, both, or neither as would be the case in chemical storage and/or hazardous waste holding areas.
- iii. Inspection Form.
- 1) Biological Safety: include such items as training, authorization, written emergency procedures, infectious agents, recombinant DNA procedures, accident investigations, NIH/CDC guidelines, and laboratory ventilation.
 - 2) Fire and Explosion Safety: are items related compliance the National Fire Protection Association (NFPA) guidelines, including storage and handling of flammable chemicals, sprinkler systems, means of egress, and compressed gas cylinders..
 - 3) General Safety Considerations: included are questions regarding the administration of the laboratory safety program, hazard communication, personal protective equipment, and electrical safety.

g. Shipping Requirements and Limitations for Infectious Substances and Diagnostic Specimens

The World Health Organization document, “Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens” is available at the EHS web site. These guidelines are applicable to the transport of infectious substances and diagnostic specimens both nationally and internationally. They provide information for identifying and classifying the material to be transported and for its safe packaging and transport. The guidelines stress the importance of developing a working relationship between the groups involved – the sender, the carrier and the receiver – in order to provide for the safe and expeditious transport of this material.

i. Definitions

For the purpose of describing transport safety measures the terms “infectious substances” and “infectious materials” are considered synonymous. The term “infectious substances” will be used in this document.

- 1) Infectious substances – An infectious substance is defined as a substance containing a viable microorganism, such as a bacterium, virus, rickettsia, parasite or fungus, that is known or reasonably believed to cause disease in humans or animals*. With respect to packaging and transport situations, infectious substances include:

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- a) all cultures containing or suspected of containing an agent which may cause infection;
- b) human or animal samples that contain such an agent in quantities sufficient to cause infection, should an exposure to them occur due to a transport mishap;
- c) sample(s) from a patient with a serious disease of unknown cause;
- d) other specimens not included above and designated as infectious by a qualified person, e.g. a physician, scientist, nurse, etc.

* This definition is taken from the current UN Recommendations on the Transport of Dangerous Goods. Prions are not included in this definition although they are considered to be infectious agents.

2) Diagnostic specimens – A diagnostic specimen is defined as any human or animal material including, but not limited to, excreta, blood and its components, tissue and tissue fluids collected for the purposes of diagnosis, but excluding live infected animals. Diagnostic specimens resulting from medical practice and research are considered a negligible threat to the public health. Diagnostic specimens obtained from patients with suspected infectious diseases may contain limited quantities of an infectious agent. There are very few agents which may be the source of an infection as a result of a transport mishap. If exposure to the specimen due to transport mishap could result in an infection, the diagnostic specimen must be packaged, labeled and transported as an infectious substance. Diagnostic specimens collected during an investigation of an outbreak of a serious disease of unknown cause must be handled as infectious substances.

ii. Packaging, Labeling and Documentation for Transport

Because of the distinction of risks between infectious substances and diagnostic specimens, there are variations to the packaging, labeling and documentation requirements. The packaging requirements are determined by the UN and are contained in ICAO and IATA regulations in the form of Packaging Instructions (PI) 602 and 650. The requirements are subject to change and upgrade by these organizations. The current packaging requirements are described in the WHO document. UN-approved packaging systems are available commercially.

h. Radiation

Radiation problems shall be referred to the Campus Radiation Safety Officer. If an emergency exists or is thought to exist, see “Radiation Safety.”