

Laboratory Safety Manual

University of Louisville



Revised: November 2018

Purpose

The purpose of this manual is to assist laboratories in establishing safe practices while working in laboratories. It is designed to comply with the OSHA 29 CFR 1450 Laboratory Standard.

Scope

The procedures and practices in this manual applies to University of Louisville employees and students while on University- owned property and/or worksites.

Responsibilities for Laboratory Safety

The use of chemicals at the University of Louisville shall be planned and performed in a manner to ensure that a safe and healthy environment is maintained. The objective of this policy is to eliminate, or reduce to the lowest feasible level, employee exposures to chemicals used in University of Louisville laboratories. Recognizing the importance and widespread use of research involving many classes of chemicals, these guidelines will attempt to facilitate this objective by defining responsibilities and procedures for essential laboratory safety.

Chairperson:

The chair has primary responsibility for the safe management of laboratories in a department, including compliance with all applicable regulatory requirements, and shall require that all laboratories in his/her charge be safely managed. He/she may delegate to other departmental faculty or staff members the authority to oversee these activities.

As the safety and wellbeing of students, faculty, and staff come above all other considerations at the University of Louisville, the chair shall ensure that no experiment that subjects personnel to excessive risk is permitted, no matter how valuable the experimental information might be.

Faculty (Principal Investigator):

- Acquire the knowledge and information needed to recognize and control hazards in the laboratory.
- Ensure completion of the lab-specific sections of the **Chemical Hygiene Plan**, including Standard Operating Procedures for highly hazardous substances. Review these documents annually and revise as required. Ensure access to the lab-specific Chemical Hygiene Plan for all lab personnel working with hazardous chemicals.
- Ensure an **inventory of hazardous chemicals** present in the lab is maintained, and **Safety Data Sheets** are readily available (paper copy or online) to all lab personnel working with hazardous chemicals.
- Evaluate safety and health hazards connected with proposed experimental procedures, select and employ laboratory practices, engineering controls and personal protective equipment that reduce the potential for exposure to chemicals to the lowest feasible level, and plan for handling of any resultant emergencies.
- Provide information and training to those employees for whom the investigator is responsible. This training should center on health and safety hazards unique to the specific laboratory, which are not included in the scope of the basic laboratory safety training. Special emphasis shall be made for highly

hazardous substances, which require written SOP's delineating laboratory practices, engineering controls, personal protective equipment, and procedures for dealing with spills and accidents.

- Require adherence to guidelines relating to safe usage of approved apparatus, the acquisition, use, and storage of hazardous materials, and the proper disposal of hazardous waste. Supervise the safety performance of staff to ensure that required laboratory practices, engineering controls, and personal protective equipment are employed.
- Be alert to and informed of federal, state, and local regulations relating to each particular laboratory operation.
- Assist representatives of the Department of Environmental Safety (DEHS) investigating accidents.
- Arrange for immediate medical attention for personnel and report to DEHS any accident that results in:
 - injury requiring medical attention
 - fire or explosion
 - ingestion or inhalation of dangerous amounts of chemicals or poisons
 - any incident resulting in overexposure of personnel or danger of environmental contamination by chemicals.
- Obtain approval from DEHS to conduct a high-risk operation involving chemical agents.
- Investigating and reporting to DEHS any problems pertaining to operation and implementation of laboratory practices and engineering controls.

Department of Environmental Health and Safety (DEHS):

- Assist the principal investigator in the selection of laboratory practices, engineering controls, and personal protective equipment.
- Provide technical guidance to personnel at all levels of responsibility on laboratory safety.
- Provide basic laboratory safety/chemical hygiene training for University personnel.
- Perform periodic safety assessments of laboratories.
- Work with University architects (Planning, Design, and Construction) and Physical Plant personnel in evaluating design parameters for laboratory facilities.
- Perform periodic testing of laboratory chemical hoods.
- Investigate all reported accidents that result in the injury or exposure of personnel or chemical release and recommend corrective action to reduce the potential for recurrence.
- Supervise decontamination operations where accidents have resulted in significant contamination of laboratory areas.
- Provide services for the routine disposal of hazardous substances.
- Recommend to the administration the means to meet government compliance with respect to hazardous materials.
- Provide health and safety review of grant proposals involving the use of hazardous chemicals when required by University Committees or outside granting agencies.

Laboratory Personnel

- Participate in required training activities.
- Know and comply with safety guidelines, regulations, and procedures required for the tasks assigned.
- Plan and execute laboratory operations in a manner that does not constitute a hazard to themselves or their coworkers.

- Understand the selection, use, and limitations of personal protective equipment (PPE). When a procedure requires the use of PPE, it is used properly.
- Consider the safety of others in the laboratory, including visitors.
- Report unsafe conditions to the principal investigator, immediate supervisor, or DEHS.
- Know and follow emergency procedures, including the location and proper use of emergency equipment.
- Report to the principal investigator or immediate supervisor and DEHS all facts pertaining to every accident or near-miss that results, or may result in, any human injury, exposure, or the uncontained spill or release of chemicals. The primary purpose of accident investigation is accident prevention, not the assignment of blame or culpability.

General Rules for Laboratory Safety

Inspections by Regulatory Agencies

Contact DEHS (852-6670) immediately if a state or local regulatory agency arrives to inspect a University facility.

Laboratory Safety Assessments

While DEHS conducts periodic assessments of laboratories, the Principal Investigator (or designee) performs lab safety self-assessments on an annual basis. A printable version of the self-assessment is available on the DEHS website, with instructions on documenting performance of the lab safety assessment in BioRAFT.

Preventative Measures

Know the safety rules and procedures that apply to the laboratory work. Determine the potential hazards and appropriate safety precautions before beginning any new operation. Be alert to unsafe conditions and actions; call attention to them to improve conditions as soon as possible.

Equipment

- Use equipment only for its designated purpose.
- Carefully position and secure any apparatus used for hazardous reactions in order to permit manipulation without moving the apparatus until the entire reaction is complete.
- Use mechanical devices for all pipetting procedures; never mouth pipette.

Personal Safety

- Know the location and use of emergency equipment (safety shower/eyewash, fire extinguisher) in your area. Know how to obtain additional help in an emergency and be familiar with emergency procedures.
- Only use a fire extinguisher if you are trained to do so. The University prefers that you evacuate the area and allow trained individuals to fight the fire.
- Protect the face, skin, and eyes, at all times by wearing appropriate personal protective equipment (PPE) to avoid direct contact with chemicals. Remove PPE before leaving the laboratory. Do not wear lab coats or other potentially contaminated protective equipment out of the lab into elevators, during lunch breaks, or launder lab coats at home.
- Sandals, open-toed shoes, and shorts should never be worn in a laboratory in which hazardous chemicals are in use.

- All workers with long hair must tie hair away from moving, rotating equipment.
- Do not eat, drink, smoke, or apply cosmetics in the laboratory or in any location where chemicals or other hazardous agents are used or stored.
- Never drink out of laboratory glassware. Glassware that has been washed with chromic acid can retain and leach this toxic chemical into contents of the glassware. Even small amounts of chromium salts are hazardous to your health.
- Remain out of the area of a fire or personal injury unless it is your responsibility to help meet the emergency responders.
- Practical jokes or horseplay will not be tolerated at any time.
- Post emergency phone numbers near lab phones and on the lab doors.
- Conduct procedures that involve hazardous volatile chemicals or that may result in the production of aerosols or dangerous gases in a properly functioning chemical hood. If this is not feasible, call DEHS to conduct a hazard assessment.
- Consider any unlabeled chemical solution hazardous until it is identified.
- Discard chemicals that have changed in color or appearance via DEHS hazardous waste disposal.
- Allow only authorized personnel in the laboratory.
- Wash hands frequently, especially before leaving the laboratory and prior to eating.

Working Alone in Laboratories

Prior approval of the Principal Investigator is required for working alone after hours.

Generally, it is prudent to avoid working alone in a laboratory. Individuals working in separate laboratories outside of working hours should make arrangements to check on each other periodically or ask security guards to check on them. Experiments known to be hazardous should not be undertaken by a worker who is alone in a laboratory. Never work alone with acutely toxic chemicals. Contact DEHS for guidance.

Unattended Laboratory Operations

Prior approval of the Principal Investigator is required for unattended laboratory operations.

Laboratory operations involving hazardous substances are sometimes carried out continuously or overnight with no one present. It is the responsibility of the worker to design these experiments so as to prevent the release of hazardous substances in the event of interruptions in utility services such as electricity, cooling water, and inert gas. Laboratory lights should be left on, and signs should be posted identifying the nature of the experiment and the hazardous substances in use. If appropriate, arrangements should be made for other workers to periodically inspect the operation. Information should be posted indicating how to contact the responsible individual in the event of an emergency.

General Housekeeping

- Keep doors locked and closed when no one is in the laboratory and especially after working hours.
- Floors should be free of trip and slip hazards, including spilled materials.
- Minimize the number of electric cords crossing walkways. If necessary, use tape or mats to reduce the risk of tripping.
- Maintain clear passageways to all exits.
- Maintain a clear space around safety showers/eyewashes, fire extinguishers, and electrical controls.
- Sink traps and floor drain traps should be filled with water at all times to prevent the escape of sewer gases into laboratories.

- Clean work areas upon completion of an experiment or at the end of each day.
- Limit the storage of chemicals under sinks to only cleaning supplies (Bleach, soap, other disinfectants).
- Sharp or pointed tools should be properly sheathed or stored.
- Clothing should be hung in proper locations and not draped over equipment or benches.
- Limit the storage chemical containers or hazardous waste on the floor. If necessary, store in secondary containers compatible with the chemicals (Nalgene/polypropylene trays).
- Do not store boxes, equipment, etc. on top of shelving close to sprinkler heads such that it would block the dispersal of water in a fire emergency.

General Laboratory Equipment

Working in a laboratory requires the use of various types of equipment. Be familiar with the equipment before using it, including how it operates, potential hazards, its safeguards, and its maintenance.

If equipment does not operate properly or is in need of repair, immediately take it out of service. Tag the equipment to indicate the deficiency and notify the PI or laboratory supervisor. Determine that all equipment:

- has adequate controls and safeguards.
- is installed in a safe location with adequate ventilation, if required.
- is being used only for its designated purpose.
- is not used in flammable or combustible atmospheres.

Glassware

Accidents involving glassware are a leading cause of laboratory injuries. In general, be certain that you have received proper instruction before using glass equipment.

- Handle and store glassware carefully.
- Properly discard or repair damaged items.
- When inserting glass tubing into rubber stoppers or when placing rubber tubing on glass hose connections, use adequate hand protection.
- Substitute plastic or metal connections for glass ones whenever possible.
- For vacuum work, use only glassware designed for that purpose.
- When dealing with broken glass:
 - wear appropriate hand protection.
 - use a broom and dustpan to sweep small pieces.
 - package it in a rigid container (sturdy cardboard box) lined with a bag, label the box "Broken Glass," and tape shut to protect housekeeping personnel.

Refrigerators

Label all laboratory refrigerators with a 'No Food/Drink' sticker. If the unit is used to store biological materials (blood, cells, bacteria, etc.), label it with a universal biohazard sticker. If the unit is not rated to store flammable materials, label it with a 'No storage of flammable materials' sticker. All stickers are available at the Radiation Safety Office (852-5231) or DEHS main office (852-6670).

Flammable chemicals cannot be stored in conventional refrigerators. Electrical sparks from a conventional refrigerator can ignite the flammable vapors that build up inside. Two kinds of refrigerators are approved for storage of flammables:

Flammable materials refrigerator: These have no spark sources within the refrigerator cabinet. There are, however, spark sources outside the refrigerator cabinet from switches, motors, relays, etc.

Explosion-proof refrigerators: These refrigerators have all spark sources completely sealed inside and are safe for flammable atmospheres both within and outside of the refrigerator cabinet.

All materials stored in a laboratory refrigerator should be properly labeled and in closed containers. Avoid glass stoppers, aluminum foil, or Parafilm as closures for long-term storage of materials.

Heating Sources

The use of Bunsen burners and other open flames is highly discouraged. If they are necessary, they must never be left on and unattended. Always wear appropriate PPE when using a heating device. Before using any heating device:

- check to see if the unit has an automatic shutoff in case of overheating.
- note the condition of electrical cords and have them replaced as required.
- make sure it has been maintained as required by the manufacturer.

Bunsen burners, alcohol burners, or other open flames should not be used inside biosafety cabinets (BSC). When necessary, lab personnel should use touch-plate microburners equipped with a pilot light. The burner must be turned off when work is completed. Small electric 'furnaces' are available for decontaminating bacteriological loops and needles and are preferable to open flame inside the BSC.

Ultraviolet Lights

The University of Louisville no longer supports the use of UV germicidal lights in biosafety cabinets as an appropriate form of decontamination. Contact DEHS (852-6670) for more information and for the disposal of UV bulbs.

Handheld UV lamp systems are designed for limited, short-term use. Always wear appropriate PPE, especially eye protection, and ensure the unit is turned off after use.

Vacuum Systems

Observe the following general precautions when using vacuum systems:

- Every laboratory vacuum pump must have a belt guard in place when it is in operation.
- The power cord and switch, if any, must be free of observable defects.
- Use a trap on the suction line to prevent liquids from being drawn into the pump.
- If vapors are being drawn through the pump, a cold trap should be inserted in the suction line to prevent contamination of the pump oil.
- Place a pan under the pump to catch any oil drips.
- Vacuum lines leading from an experimental procedure must always be equipped with traps to prevent contamination of vacuum equipment or house lines. The output of each pump should be

vented to a properly functioning fume hood. Do not discharge into an enclosed space, such as a cabinet, as this may cause an explosion.

Determine the size range of generated particulates and choose capable filtration.

- Aqueous non-volatile: in most cases, a filter flask at room temperature will prevent liquids from contaminating the vacuum source.
- Solvents or volatile liquids: A cold trap that is large enough and cold enough to condense vapors plus a filter flask large enough to hold all possible liquids that could be aspirated. Only use liquid nitrogen in sealed or evacuated equipment and with extreme caution. A slurry of dry ice and isopropanol or ethanol is suitable for most applications.
- Corrosive, highly reactive, or toxic gases: HEPA filters or a high efficiency scrubber system.

Glassware used for vacuum distillations or other uses at reduced pressure must be properly chosen for its ability to withstand the external pressure of the atmosphere.

- Only round-bottom vessels may be subjected to vacuum unless specially designed, such as Erlenmeyer-type filtration flasks.
- Each vessel must be carefully inspected for defects such as scratches or cracks prior to use.

Because all vacuum equipment is subject to failure by implosion, enclose all vacuum operations by blast shielding or conduct in a chemical hood with lowered sash and blast shielding.

Wrap glass type Dewar flasks from top to bottom with cloth tape such as electrician's friction or Mylar tape. Large Dewar flasks encased in metal and stainless steel vacuum containers do not require wrapping.

Desiccators

Glass desiccators experience partial vacuum due to the cooling of contents. They have inherent strains due to glass thickness and the relatively flat surface of the top and bottom. Obtain the available desiccator guard made of perforated metal or use a molded plastic desiccator, which is spherical and has high tensile strength.

Centrifuges

When operating a centrifuge, check the condition of the machine and rotors before and after use. This includes proper loading, keep speeds below safe operational levels, safe stopping, removal of materials, and cleanup. Ultra centrifuges require special cleaning and maintenance to prevent surface scratching.

- Inspect tubes or containers for cracks or flaws before use.
- Avoid overfilling tubes or containers.
- Properly balance tubes or containers in the rotor.
- Close the centrifuge lid during operation and allow the rotor to stop completely before opening the lid.

If centrifuging infectious materials, wait 10 minutes after the rotor completely stops before opening the lid. If a spill occurs, use appropriate decontamination and cleanup procedures for the spilled materials.

Autoclaves

- Inspect drain strainer daily and clean as necessary.

- Fill liquid containers no more than 50-75% full.
- Loosen caps or use vented closures on bottles. Never fully tighten caps on non-vented bottles.
- Leave space between items to allow steam to circulate.
- Never use plastic bags unless the top of the bag is loosened to allow steam penetration.
- Autoclave reusable syringes and needles in a pan of disinfectant.
- Do not autoclave cellulose nitrate media, such as centrifuge tubes or nitrocellulose filters.
- Place the load in secondary containment, such as Nalgene or polypropylene or stainless steel bins. Secondary containment will serve to contain the broken vessels or ruptured bags that sometimes result from routine autoclaving.
- The addition of water to a bag of largely dry material may facilitate steam penetration, though use caution to not to create aerosols of infectious microorganisms.
- Check that chamber pressure is zero prior to removing the load.
- Allow load to cool before opening the chamber. If the chamber must be opened before it has cooled, wear a lab coat, face protection (such as a face shield), heat-insulating gloves, and closed-toe shoes.
- Stand behind the door and open it slowly. Beware of a rush of steam.
- After slow exhaust cycle, open the door and allow liquids to cool before removing.

General Chemical Safety

Safety Data Sheets (SDS, formerly MSDS)

The federal Occupational Safety and Health Administration (OSHA) Hazard Communication Standard requires manufacturers or distributors of hazardous materials to provide Safety Data Sheets (SDS) to the purchaser. The Hazard Communication Standard and Laboratory Standard require labs to obtain and maintain SDS for every chemical used in the workplace. SDS must be accessible (paper or online) to personnel during all work hours.

The following sections are in all SDS:

- Product Identification
- Hazardous Ingredients Mixture
- Physical Data
- Fire and Explosion Data
- Health Hazard Data
- Emergency and First Aid Procedures
- Reactivity Data
- Spill, Leak, and Disposal Procedures
- Personal Protection Information

Product Identification

- Name and address of manufacturer, and emergency contact information
- Product name: commercial and marketing names, as well as approved synonyms
- Chemical family: group of chemicals with related physical and chemical properties
- Formula: chemical formula
- CAS number: Chemical Abstracts Service assigned number

Hazardous Ingredients Mixture

This section describes the percent composition of the substance, listing hazardous chemicals in the product. Each hazardous component comprising 1% or more of the product must be listed. Carcinogens must be listed if they comprise at least 0.1% of the product.

Exposure limits, if available, are also provided for each hazardous component. The OSHA permissible exposure limit (PEL), National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL), and/or the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) will also be listed, if appropriate. The OSHA PEL is the regulated standard, while the others are recommended limits. The PEL is usually expressed in parts per million parts of air (ppm) or milligrams of dust or vapor per cubic meter of air (mg/m³). It is usually a time-weighted average (TWA), a concentration averaged over an eight-hour day. Sometimes, a short-term exposure limit (STEL) may be listed. The STEL is a 15-minute TWA that should not be exceeded. A ceiling limit (C) is a concentration that may not be exceeded at any time. A "skin" notation means that skin exposure is significant in contributing to the overall exposure.

Physical Data

This section outlines the physical properties of the material. This information may be used to determine conditions for exposure. The following information is usually included:

- Boiling Point (BP): temperature at which liquid changes to vapor state.
- Melting Point (MO): temperature at which a solid begins to change to liquid.
- Vapor Pressure (VP): expressed in mmHg. Higher vapor pressure materials typically evaporate more quickly.
- Vapor Density: weight of a gas or vapor compared to weight of an equal volume of air (air =1). Density greater than 1 indicates it is heavier than air, less than 1 indicates it is lighter than air. Vapors heavier than air can flow along just above ground, where they may pose a fire or explosion hazard.
- Specific Gravity: ratio of volume weight of material to equal volume weight of water (water=1).
- Solubility in Water: percentage of material that will dissolve in water.
- Appearance/Odor: brief description of the appearance and odor of the product.
- % Volatile by Volume: percentage of a liquid or solid, by volume, that evaporates at a temperature of 70F.
- Evaporation Rate: the rate at which a material evaporates when compared to a known material's evaporation rate.
- Viscosity: measurement of the flow properties of a material.
- Other Pertinent Physical Data: information such as freezing point, as appropriate.

Fire and Explosion Hazard Data

This section includes information regarding the flammability of the material and information for fighting fires involving the material.

- Flashpoint: the lowest temperature at which a liquid gives off enough vapor to ignite when a source of ignition is present.
- Auto-ignition Temperature: the lowest temperature at which a flammable gas-air mixture will ignite without spark or flame.

- Flammable Limits: the lower explosive limit (LEL) and upper explosive limit (UEL) define the range of concentration of a gas or vapor in air at which combustion can occur.
- Extinguishing Media: appropriate fire extinguishing agent(s) for the material.
- Fire-fighting Procedures: Appropriate equipment and methods for limiting hazards encountered in fire situations.
- Fire or Explosion Hazards: Hazards and/or conditions that may cause fire or explosions.

Health Hazard Data

This section defines the medical signs and symptoms that may be encountered with normal exposure or overexposure to this material or its components. Information on the toxicity of the substance and route of entry may also be presented. Results of animal studies are most often given. i.e. LD50 (mouse)=250 mg/kg. Health hazard information may also distinguish the effects of acute (short term) and chronic (long-term) exposure.

Emergency and First Aid Procedures

Based on the toxicity of the product, degree of exposure, and route of contact (eye, skin, inhalation, ingestion, and injection), emergency and first aid procedures are recommended in this section.

Reactivity Data

This section includes information regarding the stability of the material and any special storage or use considerations.

- Stability: "unstable" indicates that a chemical may decompose spontaneously under normal temperatures, pressures, or mechanical shocks. Rapid decomposition produces heat and may cause fire or explosion. Conditions to avoid are listed in this section.
- Incompatibility: certain chemicals, when mixed, may create hazardous conditions. Incompatible chemicals should not be stored together.
- Hazardous Decomposition Products: chemical substances that may be created when the chemical decomposes or burns.
- Hazardous Polymerization: rapid polymerization may produce enough heat to cause containers to explode. Conditions to avoid are listed in this section.

Spill, Leak and Disposal Procedures

This section outlines general procedures, precautions and methods for cleanup of spills. Appropriate waste disposal methods are provided for safety and environmental protection.

Personal Protection Information

This section includes general information about appropriate personal protective equipment for handling this material. Many times this section of the SDS is written for large scale use of the material. Appropriate personal protection may be determined by considering the amount and use of the material. Personal protective equipment includes eye protection, skin protection, respiratory protection, and ventilation considerations.

Compressed Gas Cylinders

Laboratories utilize many gases, including those that are:

- Flammable: any gas for which flammable limits in air are reported.
- Toxic: any gas that has an LC50° less than or equal to 5000 ppm.
- Asphyxiant: covers all inert gases, toxicity caused by oxygen-deficient levels.
- Oxidizer: gases that in the presence of an ignition source and fuel support/accelerate combustion.
- Corrosive: most gases are not corrosive in the absence of water. However, since gases are released into moist air, the potential for corrosive hazard must be considered.
- Extremely cold: cryogenic liquids.

If released into the laboratory, these gases may create hazards such as depletion of oxygen, fire, and/or adverse health effects.

Falling is the most likely accident with a gas cylinder. This may cause a painful physical injury, but serious consequences result if the valve is knocked off the cylinder. The gas is rapidly released and the cylinder becomes a potentially lethal and highly destructive projectile. Personnel, even those in adjacent laboratories, may be harmed or killed if in the path of the cylinder.

Follow these rules for the safe use of cylinders.

Always:

- Secure empty and full cylinders in racks or holders, or with clamping devices.
- Secure using devices that are non-combustible (metals clamps, chains, or flame-retardant straps) and will not melt in case of a fire.
- Check the label for proper contents before using a cylinder for any purpose. Color-coding by suppliers may vary.
- Close valves and relieve pressure on the regulator when cylinders are not in use. Keep cylinders capped when not in use.
- Keep a minimal number of cylinders on hand.
- Store empty cylinders as if they were full. Identify full and empty cylinders as such.
- Prevent sparks, flames, electrical apparatus, or circuits from coming into contact with cylinders.
- Use only regulators approved for the specific gas.
- Use only oxygen-compatible threading compounds such as Teflon tape on valve threads for oxygen service.
- Protect cylinders from abnormal mechanical shocks, which may cause damage when cylinders are moved.
- When opening a cylinder valve, open slowly and direct the opening away from personnel.
- Remove the cylinder to open space and away from any possible source of ignition if a cylinder develops a leak at the valve, or if a leak occurs in any safety device.
- Store flammable gases in well-ventilated areas away from oxidizers, open flames, sparks, and other sources of heat and ignition.
- If using corrosive gases, ensure emergency showers/eyewashes are nearby.

Never:

- Move a gas cylinder unless using an appropriate cart or hand truck, the cylinder is secured to the cart, and the cap is in place.

- Store a cylinder in a hallway.
- Empty a cylinder by off-gassing it.
- Use oil, grease, or other lubricants on the regular valves or fittings.
- Use oxygen as a substitute for compressed air.
- Lift cylinders by the cap, except with an approved cylinder cart designed for this purpose.
- Tamper with, attempt to repair, or replace safety devices on cylinder valves.
- Change a cylinder's contents by refilling with a different gas.
- Use direct flames or heat lamps to raise the pressure of a cylinder.
- Use wrenches on valves equipped with a hand wheel. If the valve is faulty, segregate the cylinder for return to the manufacturer.
- Direct a gas stream toward any person.
- Force a valve connection that does not fit. Threads on regulator connections or other auxiliary equipment shall match those on the container valve outlet.
- Abandon a gas cylinder, unsecured, in a hallway or at a loading dock.

Cryogenic Safety

A cryogenic material is any substance that must be cooled to a temperature of -130C or lower to change from a gas to a liquid. Cryogenics have several distinguishing characteristics:

- Extremely cold (-120 to 270C).
- Primary cooling mechanism is vaporization (latent heat).
- High expansion ratio (averaging 800:1) when the physical state changes from liquid to vapor/gas.

Any employee using cryogenics should have a thorough knowledge of procedures, operation of equipment, safety devices, properties of materials, and use of personal protective equipment.

- Careful clean all equipment and system components.
- Strictly control mixing of gases or fluids to prevent the formation of flammable or explosive mixtures. Take extreme care to avoid contamination of a fuel with an oxidant or the contamination of an oxidant with fuel.
- Consider the properties of the gas involved when venting storage containers and lines. Always vent large storage vessels outdoors to prevent an accumulation of flammable, toxic, or inert gas in the work area. Smaller, lab sized containers can be vented into a chemical hood system.

Cryogenic fluids are usually stored in properly insulated containers designed to minimize the loss of product due to boil-off.

- A Dewar flask is the most common container for cryogenic fluids. It is a double-walled, evacuated container made of metal or glass, with a vacuum between the walls.
- Larger quantities of cryogenic fluid require double-walled metal containers of evacuated construction.
- Tape exposed glass to minimize the flying glass hazard if the container should break or implode.
- Transfer liquids from the metal Dewar vessels with special transfer tubes or pumps designed for that particular application.

Cryogenics present many hazards that may all be present concurrently.

All cryogenics can cause cold burns or frostbite when in contact with human skin.

- Do not overfill containers.
- Never make direct contact with cryogenic liquids, uninsulated cryogenic pipes, or equipment.
- Stay out of the path of boil-off gases.
- Wear suitable personal protective equipment when handling any object that may be cold.
- Transfer or pour cryogenics slowly to minimize boiling and splashing.
- Use a phase separator or special filling funnel when filling a Dewar or transferring cryogenics.
- Secure all secondary containers when filling.
- Position Dewars so the pressure relief valves and rupture disk vent paths are directed away from personnel, critical equipment or designated work areas.
- Inspect and maintain cryogenic equipment, and remove equipment from service when it does not meet manufacturer's operating specifications.
- Carry cryogen-containing Dewars with both hands and as far away from you as possible.

Fire and explosion are hazards associated with cryogenics. The source and hazards are:

- Hydrogen, methane, and acetylene, where the gases themselves are flammable.
- Oxygen. Its presence will increase the flammability of ordinary combustibles. Keep all organic materials and other flammable substances away from contact with oxygen.
- Liquefied inert gases. Liquid nitrogen and helium can condense oxygen from the atmosphere, causing oxygen entrapment in unsuspected areas.
- Extremely cold surfaces can condense oxygen from the atmosphere.

The hazard from high-pressure gas is always present with cryogenic fluids.

- Since liquefied gases are usually stored at or near their boiling point, there is always some gas present in the container.
- The large expansion ratio from liquid to gas causes a buildup of high pressure due to evaporation of the liquid.
- Utilize pressure relief devices sized for maximum possible backpressure. Inspect at regular intervals for leakage, frosting, and dirt accumulation.

Asphyxiation can occur if off gassing cryogenic liquids displace enough oxygen in a space.

- Ensure an oxygen alarm is present in the work area when appropriate.
- Periodically test and calibrate the oxygen alarm. If an oxygen monitor alarm goes off while you are in the work area, evacuate the room immediately. If it goes off in another room, do not enter the room.
- Use natural ventilation and chemical hoods where appropriate.
- Avoid transporting containers in enclosed spaces such as elevators.

Carefully select materials for cryogenic service. Drastic changes in the properties of materials occur when exposed to extremely low temperatures. Suitable metal materials include stainless steel (304 and other austenitic series), copper, brass, bronze, Monel, or aluminum. When dealing with non-metals, consider the chemical reactivity between the fluid or gas and the storage containers. Suitable non-metals include Dacron, Teflon, and Kel-F.

Flammable and Combustible Liquids

This standard acknowledges the 2000 Kentucky Fire Prevention Code that references NFPA 30 and NFPA 45. University laboratories are classified as Class B (Moderate Fire Hazard) and the following listed quantities comply with that classification. The following applies to laboratories with fire suppression systems (sprinklers). For non-sprinkler laboratories, contact the University Fire Marshal at 852-6111.

- The total amount of solvents within the laboratory shall not exceed ten (10) gallons per 100 sq. ft.
- The total amount of unprotected solvents within the laboratory shall not exceed five (5) gallons per 100 sq. ft.
- Solvents in excess of the amounts listed in item #1 shall be in bulk storage rooms meeting NFPA 30.
- Flammable solvents (i.e., flash point < 100°F) must not be stored in an ordinary refrigerator. Must use a flammable material storage refrigerator or explosion proof refrigerator.

Recommended practices:

- Limit glass containers to the smallest practical size.
- Transfer solvents in a laboratory hood or an approved bulk storage room.
- Use rubber carboys to carry glass containers of liquid.
- Store all 5-gallon metal cans in an approved flammable liquid storage cabinet or in an approved bulk storage room.
- Store glass containers not in use in flammable liquid storage cabinets.

Corrosive Materials

The term "corrosive" refers to substances that rapidly attack skin, eyes, and other living tissue. Inhaling the vapors of corrosive chemicals can also cause severe bronchial irritation or pulmonary edema. Such chemicals include strong acids (sulfuric, nitric, hydrofluoric), bases (sodium hydroxide, ammonia), dehydrating agents (sulfuric acid, sodium hydroxide, phosphorous pentoxide, calcium oxide), and oxidizing agents (picric acid, chromic acid, perchloric acid, peroxides, nitrates, nitrites).

Suitable facilities for quick drenching or flushing of the eyes and body must be located within 10 seconds walking time from the work area for immediate emergency use. Such facilities shall comply with ANSI Z358.1-1990, "Standard for Emergency Eyewash and Shower Equipment." Emergency eyewash/shower facilities must be clearly visible and unobstructed so that the unit is immediately available. Lab personnel should be familiar with the location of this equipment. Physical Plant will test all units monthly.

An eyewash station must be able to deliver ample flushing to both eyes simultaneously at 4 liters per minute for a minimum of 15 minutes. Because bottled eyewash solutions cannot meet this requirement, they are only a supplement to, but not a substitute for, plumbed eyewash stations. If a bottled eyewash is available, routinely replace the wash solution (e.g. every six months) to keep bacterial growth in check.

Always wear appropriate personal protective equipment, including a lab coat, long pants/skirt and close-toed shoes, nitrile gloves, and safety goggles/face shield.

In the event of an exposure to a corrosive chemical, immediately remove contaminated clothing, wash the affected area with copious amounts of water, and get first aid or medical help.

When diluting an acid, always add acid to water to reduce the reactive effect. Do not mix concentrated acids and bases together or use cork/rubber stoppers with strong oxidizing agents.

To properly store corrosive chemicals, consider these guidelines:

- Strong oxidizers present fire and explosion hazards when in contact with organics.
- Store in glass containers away from organic, flammable, dehydrating, or reducing agents.
- Separate acids from bases and flammables.
- Store all corrosives on a shelf below eye level, and avoid storage on the floor.

Hydrofluoric Acid (HF)

Hydrofluoric acid (HF) is a strong inorganic acid with special chemical properties that make it especially hazardous to laboratory researchers. Accidental exposures to HF can cause severe surface burns, deep tissue burns, and possibly lead to life-threatening systemic poisoning. Because of its physiologically aggressive properties, HF should only be used in a laboratory setting by laboratory personnel who are trained in its proper handling techniques, associated hazards, and emergency response procedures. A complete HF emergency response procedure is provided for guidance and training of employees and should be posted near the work area for quick reference in the event of an emergency.

Hydrogen fluoride (anhydrous hydrofluoric acid) and its solutions (hydrofluoric acid) are clear, colorless, non-combustible, highly corrosive liquids. It readily reacts with glass, ceramics, concrete, rubber, and some metals. Even though HF is not a combustible substance, reactions of HF with certain metals may produce explosive hydrogen gas. Hydrofluoric acid concentrations over 40% produce a pungent, acrid, irritating odor. Dilute HF concentrations (< 40%) do not produce significant odors or vapors unless heated.

Hydrofluoric acid is extremely corrosive to body tissues. Most HF exposures initially involve the skin, eyes, lungs, or mucous membranes. Tissue exposures to HF concentrations greater than 50% produce immediate, severe, deep-seated, slow healing burns. HF burns differ from other acid burns because the fluoride ion is able to penetrate the skin readily, resulting in deep tissue layer destruction. On the skin, these burns present initially as painful areas of white discoloration that usually proceed to form blisters.

Exposures to HF concentrations less than 50% often do not produce immediate, detectable symptoms; however, as time passes the exposed tissues may develop painful, red, and sometimes blistered areas. Skin exposures to HF concentrations between 20% and 50% typically do not show burn symptoms for 1 to 8 hours. Skin exposures to HF concentrations less than 20% may not show burn symptoms for up to 24 hours. Exposures to concentrations as low as 2% have been reported to cause symptoms.

Because the fluoride ion readily penetrates tissue, fluoride concentrations in the body can rapidly reach toxic levels if exposed to HF. Systemic fluoride toxicity can result in various serious medical conditions such as abnormal mineral storage/utilization, enzyme inhibition, blood clotting defects, metabolic pathway interruption, and abnormal heart function. The ability of HF fluoride ions to bind calcium in body tissue can result in a potentially fatal condition called hypocalcemia (markedly reduced blood concentrations of calcium).

Work Practices and Safety

The specialized hazards associated with hydrofluoric acid warrant specialized work practices and safety procedures. Carefully reviewing and following these guidelines will help prevent accidents and exposures.

1. Everyone working in a laboratory where HF is used should know its hazards and special emergency response procedures. This includes personnel who do not work directly with HF.
2. Avoid using HF when working alone. It is important to have someone nearby who knows you are working with HF and knows what to do in case of an emergency.
3. Proper personal protective equipment must be worn when working with HF, including:

- Chemical splash goggles
 - Long pants and close-toed shoes
 - Long-sleeved lab coat and/or acid resistant splash apron
 - Neoprene gloves (best protection) or nitrile gloves (short duration protection only). Neoprene gloves will provide the best protection, but nitrile gloves may be used for short duration experiments or emergencies if changed frequently. Never use latex gloves with HF for any reason.
4. Laboratories using HF should have the Hydrofluoric Acid Emergency Response Procedure document and an SDS posted and readily visible in areas of use.
 5. An emergency eyewash and shower must be nearby. Make sure you know where they are and that they are operational before starting any work with HF.
 6. Have calcium gluconate gel on hand when working with HF. Check the gel expiration date before starting any work. Everyone working in an HF-use laboratory must know when and how calcium gluconate gel is used. For further information on calcium gluconate gel, see the Emergency Response Procedure listed in the next section. If you need information on purchasing the gel, please contact DEHS 852-6670.
 7. Always work with HF in a chemical hood. Be sure the hood is operating properly and has a current airflow inspection sticker.
 8. HF should be stored in chemically compatible, properly labeled containers and separated from alkalis, metals, oxidizers, cyanides, glass, reducing materials, and sulfides. Use only chemically compatible containers when using or storing HF (polyethylene, Teflon, etc.). Remember that glass, metal, and ceramic containers are not compatible with HF. Secondary containers constructed of polyethylene are advised, especially during transport.
 9. HF should be disposed of via the DEHS online waste disposal form. If you have questions concerning waste disposal, call the Hazardous Waste Coordinator at 852-2956.
 10. In the event of an HF spill:
 - Notify those in the immediate area that a spill has occurred.
 - Evacuate nonessential personnel from the spill area.
 - Turn off ignition and heat sources if safe to do so.
 - Attend to persons who may have been exposed to acid (see next section).
 - Notify your supervisor **and** Public Safety at 852-6111. Because of the special health hazards, medical personnel must evaluate anyone exposed to HF.

Emergency Response Procedure for Hydrofluoric Acid

Because of the special health hazards associated with hydrofluoric acid, it is important that the victim seek medical care even if the exposed area is small or the acid is dilute. Time is critical. Do not delay medical treatment!

Skin Contact

1. Move victim immediately under an emergency shower or other water source and flush affected area with large amounts of water. Remember to start flushing before removing clothing. Speed and thoroughness in washing is critical.
2. Carefully remove all contaminated clothing while continuing to flush affected area with water.
3. Continue to rinse affected, unclothed area for 5 minutes. While victim is being rinsed, someone should:

- Contact Public Safety at 852-6111.
 - State there has been a person exposed to hydrofluoric acid.
 - State their location.
 - Public Safety should arrange for subsequent transport to a medical facility.
 - Obtain a hydrofluoric acid SDS and send it with victim to medical facility
4. Immediately after washing, start massaging 2.5% calcium gluconate gel into the affected skin area. Wear Neoprene or nitrile gloves (not latex) while applying the gel to prevent possible secondary exposures. Liberally apply gel often and massage the burn site continuously.
 5. While affected areas are being treated with calcium gluconate gel, the victim should be thoroughly examined for other burn sites that may have been overlooked.
 6. Medical personnel should see the victim for follow-up care as soon as possible. During transport to medical facility or while waiting for emergency response, continue massaging burn sites with calcium gluconate gel. Try to keep burned areas elevated while in transport.

Eye Contact

1. Move victim immediately to an emergency eyewash station and flush eyes gently with large amounts of water for at least 15 minutes. To aid in thorough cleansing, hold eyelids open and away from the eye while washing.
2. If the victim is wearing contact lenses, have the victim remove them if possible. Removal of contact lenses should not delay or interrupt flushing.
3. While victim's eyes are being flushed, someone should:
 - Contact Public Safety at extension 6111.
 - State there has been a person with an eye exposure to hydrofluoric acid.
 - State their location.
 - Public Safety should arrange for subsequent treatment or emergency response.
 - Obtain a hydrofluoric acid SDS and send it with victim to medical facility.
4. Medical personnel, preferably an eye specialist, should see the victim as soon as possible. During transport to medical facility, ice water compresses may be gently applied to the eyes.
5. Do not use 2.5% calcium gluconate gel in eyes.

Inhalation of Vapors

1. Move victim immediately to an area with fresh air. Keep victim calm and comfortable.
2. While victim is breathing fresh air, someone should:
 - Contact Public Safety at extension 6111.
 - State there has been a person who has inhaled hydrofluoric acid vapor.
 - State their location.
 - Public Safety should arrange for subsequent treatment or emergency response.
 - Obtain a hydrofluoric acid SDS and send it with victim to medical facility.
3. Medical personnel should see the victim as soon as possible.

Ingestion

1. If the victim is conscious, have them immediately drink large amounts of water as quickly as possible. This may help to dilute the acid. Milk or an antacid tablet taken with water may also help in providing an antidote effect.
2. While the victim is ingesting water, someone should:
 - Contact Public Safety at extension 6111.
 - State there has been a person who has ingested hydrofluoric acid.
 - State their location.
 - Public Safety should arrange for subsequent treatment or emergency response.
 - Obtain a hydrofluoric acid SDS and send it with victim to medical facility.
3. Medical personnel should see the victim immediately. HF ingestion is a life-threatening emergency.

Reactives

State and federal statutes define the reactivity of materials and regulations regarding storage and disposal. Definitions of reactivity include one or more of the following:

- Normally unstable and readily undergoes violent change without detonating.
- Reacts violently or forms potentially explosive mixtures with water.
- When mixed with water, generates toxic gases, vapors, or chemicals in a quantity sufficient to present a danger to human health or the environment.
- Cyanide or sulfide bearing waste which, when exposed to pH conditions between 2 and 12.5, can generate toxic gases, vapors, or chemicals in quantity sufficient to present a danger to human health or the environment.
- Capable of detonating or exploding when subjected to a strong initiating source or if heated under confinement.
- Readily capable of detonation or explosive decomposition or reaction at standard temperature and pressure

Reactive chemical groups include:

- Pyrophoric: readily oxidized, will ignite spontaneously in air. Examples include silane, diborane, lithium hydride, white phosphorus.
- Water-Reactive: react violently with water. Examples include the alkali metals, aluminum chloride, phosphorus pentachloride, phosphorus pentoxide, all hydrides.
- Oxidizing: react vigorously at ambient temperatures when in contact with reducing materials or may evolve oxygen at room temperature under slight heating. Examples include chlorates, permanganates, nitrates, peroxides.
- Reducing: react vigorously at ambient temperatures when in contact with oxidizing materials. They are compounds that remove oxygen from other compounds or lower valence states, often with vigorous evolution of heat. Examples include all hydrides, all acetylides, alcohols.
- Shock-Sensitive: explosive at standard temperature or if heated under confinement. Highly reactive chemicals with explosive properties require special storage, handling, and disposal procedures. Examples include picrates, azides, polynitro compounds, peroxides.

Use and storage of reactive chemicals requires expert knowledge and planning. Consult DEHS 852-6670 for specific considerations.

Shock/Heat Sensitive Chemicals

These highly reactive chemicals with explosive properties require special storage, handling, and disposal procedures. Consult DEHS 852-6670 for specific considerations.

Peroxides and Peroxide-Forming Chemicals

Organic peroxides are a special class of compounds with unusual stability problems. Specific chemicals have a strong tendency to form peroxides on exposure to air. Peroxides are hazardous because of their great sensitivity to shock, sparks, heat, or friction. Some common laboratory chemicals that are capable of forming explosive levels of peroxides include diethyl ether, methyl isobutyl ketone, 2-propanol, tetrahydrofuran (THF), dioxanes, and acetaldehyde.

A wide variety of organic compounds spontaneously form peroxides by a free radical reaction of the hydrocarbon with molecular oxygen. Under normal storage conditions, formed peroxides can accumulate in the chemical container and may explode when subjected to heat, friction, or mechanical shock. For this reason, it is imperative that all researchers learn to recognize and safely handle peroxide-forming compounds.

Recommended storage limits and specific guidelines for common peroxide-forming chemicals are listed in **Table 1**. The chemicals in List A "**peroxide hazard on storage - without concentration**" of the table can form peroxides that are difficult to detect and eliminate. These peroxides can come out of solution and form crystals or a gel in the bottom of the container. They are extremely unstable and can violently decompose with the smallest disturbance, sometimes even spontaneously. They can be hazardous even if not opened. Do not store these chemicals more than 12 months unless tests show that they contain less than 80 ppm of peroxides.

The chemicals in List B "**hazard due to peroxide concentration**" can undergo explosive polymerization initiated by dissolved oxygen. This class of peroxide-forming chemicals has a propensity for exploding when used experimentally in operations such as distillations. Do not store these chemicals more than 12 months unless tests show that they contain less than 80 ppm of peroxides.

The chemicals in List C "**autopolymerize as a result of peroxide accumulation**" may explode when relatively small quantities of peroxides form. It is common to distill these peroxide-forming solvents before use, concentrating the dissolved peroxides and subjecting them to heat and mechanical shock.

Table 1: Storage limits for common chemicals that form explosive levels of peroxides

Form potentially explosive peroxides without concentration. Dispose or test 3 months after opening.	Form potentially explosive peroxides on concentration. Dispose or test 12 months after opening.	Autopolymerize as a result of peroxide accumulation. Dispose or test inhibited materials after 12 months.
Butadiene	Acetal	Acrylic acid
Chloroprene	Acetaldehyde	Acrylonitrile
Divinyl acetylene	Benzyl alcohol	Butadiene
Tetrafluoroethylene	Isopropyl ether	2-butanol
Vinylidene chloride	Cyclohexanol	Chlorotrifluoroethylene

Methacrylate	2-cyclohexen-1-ol	Methyl
	Cumene	Styrene
	Decahydronaphthalene	Vinyl acetate
	Diacetylene	Vinyl acetylene
	Dicyclopentadiene	Vinyl chloride
	Diethyl ether	Vinyl pyridine
	Diethylene glycol	
	Dimethyl ether	
	Dioxanes	
	Ethylene glycol dimethyl ether	
	4-haptanol	
	Methyl acetylene	
	Methyl isobutyl ketone	
	3-methyl-1-butanol	
	Methyl cyclopentane	
	2-pentanol	
	4-penten-1-ol	
	1-phenylethanol	
	2-phenylethanol	
	2-propanol (isopropanol, IPA)	
	Tetrahydrofuran (THF)	
	Tetrahydronaphthalene	
	Vinyl ethers	
	Other secondary alcohols	

Table 2 displays other peroxide-forming chemicals that cannot be placed into these categories but require handling with precautions.

Table 2: Possible peroxide-forming chemicals

Chemical name	Chemical name	Chemical name	Chemical name
Acrolein	tert-Butyl methyl ether	Di(1-propynyl) ether	n-Methylphenetole
Allyl ether	n-Butyl phenyl ether	Di(2-propynyl) ether	2-Methyltetrahydrofuran
Allyl ethyl ether	n-Butyl vinyl ether	Di-n-propoxymethane	3-Methoxy-1-butyl acetate
Allyl phenyl ether	Chloroacetaldehyde diethylacetal	1,2-Epoxy-3-isopropoxypropane	2-Methoxyethanol
p-(n-Amyloxy)benzoyl chloride	2-Chlorobutadiene	1,2-Epoxy-3-phenoxypropane	3-Methoxyethyl acetate
n-Amyl ether	1-(2-Chloroethoxy)-2-phenoxyethane	p-Ethoxyacetophenone	2-Methoxyethyl vinyl ether
Benzyl n-butyl ether	Chloroethylene	1-(2-Ethoxyethoxy)ethyl acetate	Methoxy-1,3,5,7-cyclooctatetraene
Benzyl ether	Chloromethyl methyl ether	2-Ethoxyethyl acetate	b-Methoxypropionitrile
Benzyl ethyl ether	b-Chlorophenetole	(2-Ethoxyethyl)-a-benzoyl benzoate	m-Nitrophenetole
Benzyl methyl ether	o-Chlorophenetole	1-Ethoxynaphthalene	1-Octene
Benzyl-1-naphthyl ether	p-Chlorophenetole	o,p-Ethoxyphenyl isocyanate	Oxybis(2-ethyl acetate)
1,2-Bis(2-chloroethoxy)ethane	Cyclooctene	1-Ethoxy-2-propyne	Oxybis(2-ethyl benzoate)
Bis(2-ethoxyethyl)ether	Cyclopropyl methyl ether	3-Ethoxypropionitrile	b,b-Oxydipropionitrile
Bis(2-(methoxyethoxy)ethyl) ether	Diallyl ether	2-Ethylacrylaldehyde oxime	1-Pentene
Bis(2-chloroethyl) ether	p-Di-n-butoxybenzene	2-Ethylbutanol	Phenoxyacetyl chloride
Bis(2-ethoxyethyl) adipate	1,2-Dibenzoyloxyethane	Ethyl-b-ethoxypropionate	a-Phenoxypropionyl chloride
Bis(2-methoxyethyl) carbonate	p-Dibenzoyloxybenzene	2-Ethylhexanal	Phenyl-o-propyl ether
Bis(2-methoxyethyl) ether	1,2-Dichloroethyl ethyl ether	Ethyl vinyl ether	p-Phenylphenetone
Bis(2-methoxyethyl) phthalate	2,4-Dichlorophenetole	Furan	n-Propyl ether

Bis(2-methoxymethyl) adipate	Diethoxymethane	2,5-Hexadiyn-1-ol	n-Propyl isopropyl ether
Bis(2-n-butoxyethyl) phthalate	2,2-Diethoxypropane	4,5-Hexadien-2-yn-1-ol	Sodium 8-11-14-eicosatetraenoate
Bis(2-phenoxyethyl) ether	Diethyl ethoxymethylenemalonate	n-Hexyl ether	Sodium ethoxyacetylde
Bis(4-chlorobutyl) ether	Diethyl fumarate	o,p-Iodophenetole	Tetrahydropyran
Bis(chloromethyl) ether	Diethyl acetal	Isoamyl benzyl ether	Triethylene glycol diacetate
2-Bromomethyl ethyl ether	Diethylketene	Isoamyl ether	Triethylene glycol dipropionate
beta-Bromophenetole	m,o,p-Diethoxybenzene	Isobutyl vinyl ether	1,3,3-Trimethoxypropene
o-Bromophenetole	1,2-Diethoxyethane	Isophorone	1,1,2,3-Tetrachloro-1,3-butadiene
p-Bromophenetole	Dimethoxymethane	b-Isopropoxypropionitrile	4-Vinyl cyclohexene
3-Bromopropyl phenyl ether	1,1-Dimethoxyethane	Isopropyl-2,4,5-trichlorophenoxy acetate	Vinylene carbonate

Many methods can stabilize or inhibit the peroxidation of susceptible chemicals. If it does not interfere with the use of the chemical, and if available, peroxide scavengers (inhibitors) shall be added in small quantities, and peroxide-forming chemicals shall be ordered with inhibitor added.

Storage, Labeling, and Shelf-Life Limitations

Store peroxide-forming chemicals in sealed, air-impermeable, light-resistant containers and keep away from light. Store in original manufacturer's container whenever possible. This is very important in the case of diethyl ether; the iron in the original steel containers acts as a peroxide inhibitor.

Peroxides tend to form in materials as a function of age. Therefore, it is imperative that researchers are keenly aware of the age of their peroxide-forming chemicals. Date each container upon arrival and again when opened for the first time. If not already on the label, an appropriate expiration date can be determined using **Table 1**.

Testing, Management, and Disposal

When the date on the container expires, test the peroxide-forming chemical for peroxide content or assume it contains excessive peroxides and dispose of as hazardous waste. The maximum allowable concentration of peroxide in chemicals is 80 ppm. If a value over 80 ppm is detected, the owner shall deperoxidize the chemical or dispose of it as hazardous waste. If under 80 ppm but past the expiration date, continue to test quarterly. All chemicals to be distilled must be tested prior to distillation regardless of age. Never open or test containers of unknown age or origin.

The easiest method to test for peroxides is the use of peroxide test strips. These strips can be obtained from a variety of suppliers. Keep test strips refrigerated and ensure an expiration date is on the bottle.

Older containers of peroxide-forming chemicals, or containers of unknown age or history, must be handled very carefully and should never be opened. Any peroxide-forming chemical with visible discoloration, crystallization, or liquid stratification should be treated as potentially explosive. Older steel containers that have visible rust may also be extremely dangerous. If any of these conditions are observed on a peroxide-forming chemical container, or if the origin and age are unknown, do not attempt to move or open the container. Contact DEHS 852-6670 to have the container disposed of properly.

Safe Distillation

Eliminate the peroxides with a chemical reducing agent or pass the solvent through activated alumina. Add mineral oil to the distillation pot. This has the combined effect of "cushioning" any bumping, maintaining dilution, and serving as a viscous reaction moderator in case the peroxides begin to decompose.

Reducing Peroxides during Distillation

Add small pieces of sodium metal to the distillation vessel to reduce peroxides. Use benzophenone as an indicator for the presence of sodium metal (benzophenone in the presence of sodium metal forms a radical with a deep-blue color). When the blue color disappears, add more sodium metal.

Picric Acid

The yellow crystals of picric acid, or trinitrophenol, are shock-sensitive and may readily detonate if the chemical dries. Check containers of picric acid regularly to ensure the crystals remain moist with water and have not dried.

DO NOT attempt to remove the lid of a container if the acid is dry.

Contact with copper, lead, zinc, and other metals can form picrate salts that are much more shock-sensitive than picric acid itself. Unstable salts can form with concrete, ammonium, calcium, and bases.

Picric acid is a more powerful explosive than TNT and must be handled with extreme care. Contact DEHS 852-2956 for special disposal arrangements.

Perchloric Acid

Perchloric acid is a very strong oxidizing agent that can undergo vigorous, self-sustained decomposition or explosive reactions when catalyzed or exposed to heat. The majority of accidents involving perchloric acid are due to its mixture with organic material or the formation of the anhydrous acid, which is extremely unstable and explodes upon contact with wood, paper, or organic solvents. Cold perchloric acid, at less than 70% HClO₄ by weight, is a less powerful oxidizing agent. As the percentage or the temperature increases, the oxidizing power of the perchloric acid also increases.

Combustible materials, such as wood or oil, contaminated with perchloric acid are highly flammable and dangerous. Such materials may explode spontaneously, upon impact, or when in contact with heat or flame. Other perchlorates are also sensitive to heat and shock, such as heavy metal and organic perchlorates, hydrazine perchlorate, and ammonium perchlorate.

Special Precautions

- Whenever possible, purchase 60% by weight HClO₄ grade.
- Perchloric acid should be stored on a non-metal surface away from combustible materials, organic solvents, metal hydrides, alcohols, sulfuric acid, and acetic anhydride.
- Inspect monthly for signs of discoloration that indicate development of the anhydrous form of perchloric acid. Contact DEHS 852-2956 for immediate disposal.
- Wear goggles and rubber gloves whenever handling perchloric acid.
- To avoid a violent reaction in procedures requiring perchloric acid for wet digestion, pretreat the sample with nitric acid to destroy oxidizable material.
- Procedures involving heated perchloric acid require use of a special chemical hood, termed a perchloric acid hood. This type of hood is constructed of stainless steel, has a duct wash down system, and has no exposed organic coatings or sealing components. There is currently only one perchloric acid hood at the University of Louisville. Contact DEHS 852-6670 for guidance on use.

Azides and other Explosive Chemicals

The azide group of chemicals represents one of the few commercially produced explosives containing no oxygen. When compounded with other materials, azides are shock- and heat-sensitive. Copper and lead azides are more sensitive primary explosives than nitroglycerine. Sodium azide reacts with copper, lead, brass, or solder to form these explosive combinations.

In general, other compounds tend to be heat- and shock-sensitive if they contain acetylide, diazo, nitroso, or organic nitrate functional groups. Hydrogen and chlorine gases react explosively in the presence of light. Hydrazine can explode in contact with iron rust.

Toxic Chemicals

Many chemicals display more than one type of hazard. Highly hazardous chemicals require special written procedures to ensure safe use in the laboratory.

Toxicity is the potential of a substance to produce an adverse reaction on the health or well-being of an individual. Whether or not any ill effects occur depends on properties of the chemical, the route of exposure, the dose, and the susceptibility of the exposed individual.

There are four main routes of exposure:

- Inhalation
- Ingestion
- Absorption through the skin
- Injection

When a toxic chemical acts on the human body, the nature and extent of the adverse reaction depends on the dose received (amount and time interval). Two standardized measurements, the LD₅₀ and LC₅₀, serve to quantify and express the degree of toxicity of a substance.

LD₅₀ - The quantity of a material that when ingested, injected, or applied to the skin as a single dose will cause death of 50% of test animals.

LC₅₀ - The concentration of a substance in the air that causes death of 50% of test animals.

Acute toxic effects are usually produced by a single large dose received in a short period of time (usually less than 24 hours) and involve immediate results of some kind.

Chronic toxic effects are usually produced by long-duration or repeated exposure to a substance. Damage to the body may not appear for many years since chronic toxins can have long latency periods.

Organic Solvents

Organic solvents are one of the most commonly encountered groups of toxic chemicals in a laboratory. Most are highly volatile or flammable such as ethers, alcohols, and hydrocarbons. Chlorinated solvents such as chloroform are often non-flammable but, when exposed to heat or flame, may produce carbon monoxide, chlorine, phosgene, or other highly toxic gases.

Inhalation of solvent vapors may cause bronchial irritation, dizziness, central nervous system depression, nausea, headache, or coma. Prolonged exposure to high concentrations of solvent vapors may result in liver or kidney damage. Skin contact may produce defatting and drying. Ingestion of a solvent could result in severe physiological effects. In case of ingestion, call the Poison Control Center (589-8222), or seek medical aid immediately.

For some chemicals, the odor threshold is higher than the acceptable exposure limit. Examples include chloroform, benzene, carbon tetrachloride, and methylene chloride.

Certain solvents are known or suspected to be carcinogenic following prolonged exposure. See the section on Highly Hazardous Chemicals for special requirements for carcinogens. Examples include:

- chloroform
- benzene
- carbon tetrachloride
- chlorinated ethers
- methylene chloride
- polyhalogenated hydrocarbons

All volatile and flammable solvents should be used in a properly functioning chemical hood. Never use ether or other highly flammable solvents in a room with an open flame or other ignition source present.

Irritants

Irritants are non-corrosive chemicals that cause reversible inflammatory effects (swelling and redness) on living tissue at the site of contact. Many organic and inorganic chemicals are irritants. Examples include acetaldehyde, acetic acid, acrolein, ammonia, ethylene glycol, glutaraldehyde, sodium hydroxide, and xylenes.

Allergens

A chemical allergy is an adverse reaction by the immune system to a chemical. Such allergic reactions result from previous sensitization to that chemical or a structurally similar chemical. Once sensitization occurs, allergic reactions can result from exposure to extremely low doses of the chemical. Allergic reactions can be immediate, occurring within a few minutes after exposure. Anaphylactic shock is a severe immediate allergic reaction that can result in death if not treated quickly. Allergic reactions can also be delayed, taking hours or even days to develop. The skin is usually the site of such delayed reactions, in which case it becomes red, swollen, and itchy.

It is important to recognize that a delayed chemical allergy can occur even some time after the removal of the chemical. Individuals may also exhibit wide differences in their sensitivity to laboratory

chemicals. Examples include diazomethane, dicyclohexylcarbodiimide, formaldehyde, various isocyanates, benzylic and allylic halides, and certain phenol derivatives.

Latex allergy can result from repeated exposures to proteins in natural rubber latex through skin contact or inhalation. Once sensitized, workers may experience the effects of latex allergy. Symptoms include skin rash and inflammation, respiratory irritation, asthma, and in rare cases shock.

Consider the following work practices to reduce the risk of allergic reactions:

- Wear non-latex gloves for tasks that are not likely to involve contact with infectious materials such as blood.
- Follow appropriate work practices. Always wash hands with a mild soap and dry thoroughly after removing latex gloves. Identify and clean areas contaminated with latex-containing dust. Frequently change ventilation filters and vacuum bags used in those areas.
- Educate and train workers about latex allergy.
- Consult a healthcare professional if symptoms occur.
- If there is a known latex allergy, discuss options with laboratory management.

Highly Hazardous Chemicals

The OSHA Lab Standard specifically lists three categories of chemicals as particularly hazardous: include select carcinogens, reproductive toxins, and substances with a high degree of acute toxicity.

Select Carcinogens

Chemicals strongly implicated as a cause of cancer are termed carcinogenic. Substances defined by OSHA as select carcinogens fall into one of the categories listed below.

- OSHA Carcinogen: a chemical regulated by OSHA as a carcinogen, each with its own standard in subpart 2 of the OSHA General Industry Standards.
- Known Human Carcinogen: classified as "known to be carcinogens," in the most recent Annual Report on Carcinogens issued by the National Toxicology Program (NTP), or listed under Group 1 "carcinogenic to humans" by the International Agency for Research on Cancer (IARC)
- Potential Human Carcinogen: listed under IARC Group 2A "probably carcinogenic to humans" or Group 2B "possibly carcinogenic to humans", classified as "reasonably anticipated to be a carcinogen" by NTP, or causes statistically significant tumor incidence in experimental animals under any of the following dosage criteria:
 - Inhalation exposure: chronic exposure (for a significant portion of a lifetime); 6-7 hours/day, 5 days/week; dose <10mg/m³
 - Skin exposure: repeated skin exposure of <300mg/kg body weight per week
 - Ingestion: daily dose <50mg/kg body weight

The lack of inclusion of a chemical on one of these lists does not necessarily imply that it is free from carcinogenic activity. Substances such as ethidium bromide are used extensively in research but do not have a substantial industrial use and, consequently, have not undergone a rigorous analysis for carcinogenicity. Although ethidium bromide is a well-known potent mutagen, it is not included in any of these lists. Determine toxicity and hazard potential before use of any chemical.

Table 3: OSHA-Regulated and Select Carcinogens at University of Louisville

Chemical name	Chemical name
2-acetylaminofluorene	Ethylene Oxide
Acrylonitrile	Ethylenimine
Alpha - naphthylamine	Formaldehyde
4-aminodiphenyl	Inorganic Arsenic
Benzene	Methyl Chloromethyl Ether
Benzidine	Methylene Chloride
beta-naphthylamine	Methylenedianiline
beta-propiolactone	N-nitrosodimethylamine
Bis- chloromethyl ether	Vinyl Chloride
1,3 - butadiene	Acrylamide
Cadmium	Chromium and certain Cr compounds
Coke Oven Emissions	Cyclophosphamide
1,2 - dibromo-3-chloropropane	Estrogens, conjugated
3,3 - dichlorobenzidine	4-nitrosobiphenyl
4 - dimethylaminoazobenzene	

Chemicals of Unknown but Suspect Carcinogenic Potential

Most laboratories have a number of chemicals for which there is little epidemiological data regarding its carcinogenicity. While these compounds may not fall under the regulatory umbrella of an OSHA 'select carcinogen,' implement precautions to keep exposures as low as possible.

Reproductive Toxins

Chemicals that interfere in any way with the normal reproductive process are reproductive toxins. Adverse effects include reductions in libido, reduced fertility, embryo lethality, induction of chromosomal damage (mutations), malformations of the developing fetus (teratogenesis), and postnatal functional defects.

Pregnant lab personnel should exercise caution when handling or working with any chemicals. Notify lab management when pregnant to assess hazards and appropriate protective measures. Embryo toxins damage the fertilized egg, embryo, or fetus in the early stages of gestation. These chemicals have their greatest impact in the first trimester of pregnancy. Lab personnel of childbearing potential should be especially cautious when working with chemicals, especially those rapidly absorbed through the skin (e.g., formamide).

DEHS, in consultation with a medical professional, can assist staff in performing a hazard assessment of the workplace.

Table 4: Agents Toxic to the Reproductive System

In Men, Chemical Name	In Women, Chemical Name
Anesthetic gases	Anesthetic gases
Carbon disulfide	Aniline
Chemotherapeutic cancer agents	Benzene
Chlordecone (Kepone)	Carbon disulfide
Chloroprene	Chemotherapeutic cancer agents
Dibromochloropropane (DBCP)	Chloroprene
Dinitrotoluene	Ethanol consumption
Ethylene dibromide	Ethylene oxide
Ethylene glycol monoethyl ether	Glycol ethers
Ethylene oxide	Formaldehyde
Ethanol consumption	Inorganic lead
Glycol ethers	Organic lead
Hexane	Methylmercury
Inorganic Cadmium	Pesticides
Inorganic lead	Phthalic acid esters (PAEs)
Organic lead	Polychlorinated biphenyls(PCBs)
Female oral contraceptives	Streptomycin
Pesticides	Styrene
Tobacco smoking	Tobacco smoking
High temperatures	Toluene
Vinyl chloride	Vinyl chloride

Substances with a High Degree of Acute Toxicity

Substances with a high degree of acute toxicity are those that can cause death, disability, or serious injury after a single, relatively low-level exposure. The following table denotes the OSHA-defined toxicity designations, for various routes of exposures.

OSHA Hazard Designation	Other Toxicity Rating	Oral LD50 (rats, mg/kg)	Skin Contact LD50 (rabbits, mg/kg)	Inhalation LC50 (rats, ppm for 1 hr)	Inhalation LC50 (rats, mg/m³ for 1 hr)
Highly Toxic	Highly Toxic	< 50	< 200	< 200	< 2000
Toxic	Moderately Toxic	50 – 500	200 – 1000	200 – 2000	2000 – 20000
	Slightly Toxic	500 – 5000	2000 – 20000	2000 – 20000	20000 – 200000

Work involving highly hazardous chemicals requires lab-specific SOPs providing detailed information relevant to safety and health considerations. Find the LD50 on the SDS or in the Registry of Toxic Effects of Chemical Substances (RTECS). Compare the LD50 to the table above to determine if it is highly toxic and requiring a written SOP.

By this definition, many drugs and physiologically active compounds are highly toxic. A drug supplied in a ready-to-use formulation presents a much different level of risk than the pure, undiluted compound. The hazards presented by drugs and other physiologically active compounds in the lab are similar to those presented to pharmacists preparing drug formulations, where considerable precautions are undertaken.

Select Agents

DEHS provides the University oversight of select agents as required by federal laws & regulation. As a consequence of the 9/11 terrorist attacks, legislation was passed by Congress and signed by the President on October 26, 2001, which affects the possession of biological agents and toxins with bioterrorism potential termed "Select Agents." The legislation, entitled the USA PATRIOT Act, extends previous laws that restricted the transport but not the possession of these agents. Transfer, shipping, or receiving of any select agent within or outside the University is prohibited without DEHS approval.

Due to ongoing national concerns regarding the security and possession of "Select Agents," additional legislation entitled the Public Health Security and Bioterrorism Preparedness and Response Act was passed and signed into law on June 12, 2002. The regulations became effective February 7, 2003 and included many new provisions with significant criminal penalties that extend to individuals for failure to comply. The law requires that all persons possessing select biological agents or toxins register with the appropriate federal agency. For more information about Select Action Registration, contact DEHS 852-6670.

Hazard Control and Risk Minimization

Whenever possible, substitute highly hazardous materials with less hazardous materials. If not possible, consider modifying procedures to minimize direct manipulation of hazardous materials. Some highly hazardous materials are available in a form or dilution that reduces the need for direct manipulation.

When working with a highly hazardous chemical, it is necessary to develop a lab-specific written standard operating procedure (SOP) to outline the risks associated with the chemical as well as to how to mitigate those risks. SOPs for highly hazardous chemicals can be substance-specific or procedure-specific, depending on the needs of the laboratory. Contact DEHS 852-6670 for assistance with SOPs.

Confine operations involving highly hazardous materials to a designated work area in the laboratory. Post warning signs to indicate these areas and the nature of the hazard.

Limit access to laboratories where highly hazardous chemicals are in use to authorized personnel.

Conduct procedures involving highly toxic chemicals that can generate dust, vapors, or aerosols in a chemical hood, glove box, or other suitable containment device.

While all laboratory staff are responsible for conducting experimental procedures in a safe manner, the Principal Investigator is ultimately responsible for ensuring identification and implementation of appropriate precautions when dealing with highly hazardous materials. DEHS can assist with risk assessments for hazardous materials and procedures.

Hazardous Drugs

Pharmaceuticals manipulated at concentrations appropriate for patient therapy may pose a risk to lab personnel through acute or chronic exposures. Many hazardous drugs are known human carcinogens, particularly chemotherapeutic drugs. Treat analogues and derivatives of these agents as hazardous medications, and always consult the manufacturer's literature and package inserts for additional information. The greatest risk of occupational exposures to hazardous drugs occurs during procedures involving manipulation of materials in the pure form or surface contamination from spills.

Additional exposure concerns are present when hazardous chemicals/drugs are administered to experimental animals. Animals excrete many of these chemicals and/or metabolites in urine or feces following administration. Bedding collected during this period is contaminated and handled in a way that reduces the generation of dust and limits the exposure of animal care personnel. A Special Animal Safety Protocol (SASP) may be required for administration to animals. Contact DEHS 852-6670 for assistance with SASPs.

General Chemical Handling and Management

Use the following general guidelines when handling any chemical.

- Know the location of safety shower/eyewash stations, fire extinguishers, first aid kits, and spill kits.
- Conduct procedures involving hazardous volatile chemicals in a functioning chemical hood.
- Wear appropriate personal protective equipment and remove it before leaving the workspace.
- Do not launder lab coats at home. Consult with PI for information on lab coat services.
- Always wash hands when removing gloves and especially before leaving the laboratory.

Laboratory Chemical Hoods

The laboratory chemical hood is often the primary control device when using flammable and toxic chemicals in the laboratory.

- Confirm that the hood is operational. Check the airflow gauge and/or flow ribbon before use to ensure the hood is drawing air. Do not silence airflow alarms.
- Set sash height to the indicated optimal working height (most commonly 18 inches, indicated by yellow label).
- Maintain work at least 6 inches inside the hood face.
- Keep storage inside the hood to a minimum, including obstructions to the front and back grills, to minimize airflow disruptions.

- Minimize local foot traffic, open windows, fans, and/or incubators/refrigerators/freezers during use to reduce turbulence or escape of vapors from the hood.
- Use extreme caution with ignition sources inside a hood.
- Every component of a chemical hood is essential to its proper operation. Do not remove or alter components. Contact Physical Plant 852-6241 (Belknap) or 852-5695 (HSC) or the manufacturer for maintenance questions.
- Biological safety cabinets and tissue culture hoods are not the same as chemical hoods.
- Not all chemical hoods are appropriate for use with radioisotopes. Contact Radiation Safety 852-5231 for assistance.
- Perchloric acid hoods contain special safety features. Contact DEHS 852-6670 for assistance.

Personal Protective Equipment (PPE)

Personal protective equipment (PPE) protects personnel from injuries or illnesses resulting from contact with chemical, radiological, physical, electrical, mechanical, or other hazards. PPE is the last line of defense and not a substitute for engineering, work practice, or administrative controls. Contact DEHS 852-6670 for assistance with a PPE hazard assessment.

Eye and Face Protection

Safety glasses with side shields that conform to ANSI standard Z87.1 are required for work with hazardous chemicals. Ordinary prescription glasses are not adequate protection. Contact lenses can be worn safely if appropriate eye and face protection is also worn.

Splash goggles are more suitable if there is a chemical splash potential. Full face shields with splash goggles are recommended when handling explosives, highly hazardous chemicals, or large quantities of any chemical. Specialized eye protection is recommended for work involving lasers, ultraviolet light, infrared light, or intense visible light.

Hand Protection

Always wear gloves appropriate to the hazard. Inspect gloves for defects prior to use. Nitrile gloves offer the most general protection against chemicals, but contact DEHS for concerns about proper glove selection. Specialized gloves for cryogenic fluids or heat sources/autoclaves should be available when necessary.

Replace gloves immediately if contaminated. Do not reuse disposable gloves, and place used gloves in the proper waste receptacle. Always remove gloves and wash hands before leaving a workspace.

Clothing

Wear long pants, close-toed shoes, and a lab coat when working with chemicals. Fasten lab coats and do not roll up the sleeves.

Hearing Protection and Respirators

PPE may be necessary for loud equipment, such as sonicators. Contact DEHS 852-6670 if there is a concern about hearing protection/appropriate PPE.

DEHS must approve the use of all respirators. The OSHA Standard for Respiratory Protection mandates respiratory protection when it is required to reduce potential exposure to acceptable levels. The University of Louisville Respiratory Protection Program details requirements for respirator selection and

use, medical approval, fit testing, training, and maintenance and care of respirators. Before using a respirator, lab personnel must contact DEHS and enroll in the program.

Voluntary Use of Filtering Face Piece Respirators (Dust Masks)

Dust masks offer no protection against volatile chemicals.

Lab personnel may wish to use respiratory protection (such as dust masks), even when exposures are below the regulatory exposure limit, to provide an additional level of comfort and protection. If a dust mask is not disposed of after use or kept clean, the respirator itself can become a hazard.

Voluntary use of dust masks in non-hazardous situations does not require medical evaluation or fit testing; however, contact DEHS for assistance with providing advisory information to lab personnel on voluntary use.

Chemical Labeling and Container Condition

Label all containers containing chemicals with full chemical name and all applicable hazard classes (flammable, corrosive, toxic, etc.). Chemical names may be abbreviated on working solutions or dilutions from stock containers, but full name is recommended for long-term storage.

Keep chemical containers closed using screw-top caps whenever possible. Avoid Parafilm, foil, and glass/cork stoppers for long-term storage. Ensure caps and containers are not cracked or swelling. Keep containers clean of any residue or spills.

Chemical Storage and Segregation

Maintain a chemical inventory, most importantly recording chemical name and amount. Incompatible chemicals are those that, if inadvertently mixed, could produce toxic gases, explosive reactions, or spontaneous ignition. Segregate such chemicals to avoid potential reactions.

- Store flammable liquids in an approved flammable cabinet or appropriate refrigerator.
- Store volatile liquids in corrosive/vented cabinets.
- Segregate acids, bases, flammables, and oxidizers.
- Segregate reactive chemicals, and organic reactives from inorganic reactives (metals).
- Segregate acutely toxic chemicals.
- Avoid storing chemicals on the floor, or utilize secondary containment (Nalgene/polypropylene trays) if necessary.
- Avoid storing chemicals on shelves above eye level, and equip high shelves with lips.
- Avoid storing chemicals under sinks. Limit this space to cleaning/disinfectant products.
- Be cautious when ordering chemicals alphabetically. Segregate by hazard class first.