Rules and regulations regarding work practices are designed to help EPA employees live a safe and healthy life. It cannot be overstressed that each person is largely responsible for their own safety and health. Studies of industrial accidents show that less than 15 percent of accidents can be attributed to equipment failure alone; the other 85 percent or more involve human error.

This section contains information on many operations at EPA laboratories. These guidelines provide a basis for safe laboratory operations. Some basic rules need the full support and cooperation of all employees:

- Do not smoke in the laboratory.
- Do not pipette by mouth.
- Do not eat or drink in the laboratory.
- Wear eye protection at all times in the laboratory.
- Wear appropriate personal protective equipment.

Following these rules is essential for employee safety and health. This chapter of the manual provides the following guidance for EPA laboratories on work practice controls:

Chapter	Topic
F2	General Work Practice Controls
F3	Work Practice Controls for Chemicals

1.0 Introduction

EPA laboratory employees may encounter various hazards that can be controlled by safe work practices. Guidelines and standard operating procedures (SOPs) are designed to decrease the chance of employee chemical exposure or chemical cross-contamination. This chapter includes work practices associated with basic laboratory hygiene and general laboratory safety. These practices are developed and implemented to provide control over laboratory hazards.

EPA Program Requirements

Each laboratory must ensure that:

- Guidelines and SOPs are developed and followed for basic laboratory hygiene and general laboratory safety.
- Employees have been provided training on general work practice controls.
- Laboratories have been surveyed to determine compliance with guidelines and SOPs.

Program Administration

To effectively implement general work practice controls, responsibilities should be assigned to:

- Ensure that laboratory employees are following basic practices for food and beverages, apparel and jewelry, housekeeping, and personal hygiene.

- Review requests for performing unattended and overnight operations or working alone.

- Develop SOPs for the safe operation of laboratory equipment and laboratory electrical and mechanical safety.

- Train laboratory employees on general work practice controls.

- Perform laboratory surveillance to assess conformance with glassware and sharps handling, disinfection, and sterilization techniques.

- Ensure that proper decontamination of laboratories, equipment and personnel is being performed.

2.0 Basic Laboratory Hygiene Practices

Outlined below are many basic laboratory hygiene practices that should be implemented in all EPA laboratories to decrease the possibility of employee chemical exposure or cross-contamination. Figure F2-1 summarizes major categories covered for these practices and depicts some examples of signage.

2.1 Food and Beverages

When working in an EPA laboratory, eating, drinking, chewing gum, applying cosmetics, and handling contact lenses are not permitted. In addition, the storage of food and beverages in laboratory refrigerators or freezers is not permitted.

2.2 Apparel and Jewelry

Clothing to be worn in the laboratory must provide adequate coverage to protect against hazards. For example:

- Open-toed shoes are not permitted in laboratories.
- Long pants and long-sleeved shirts should be encouraged.

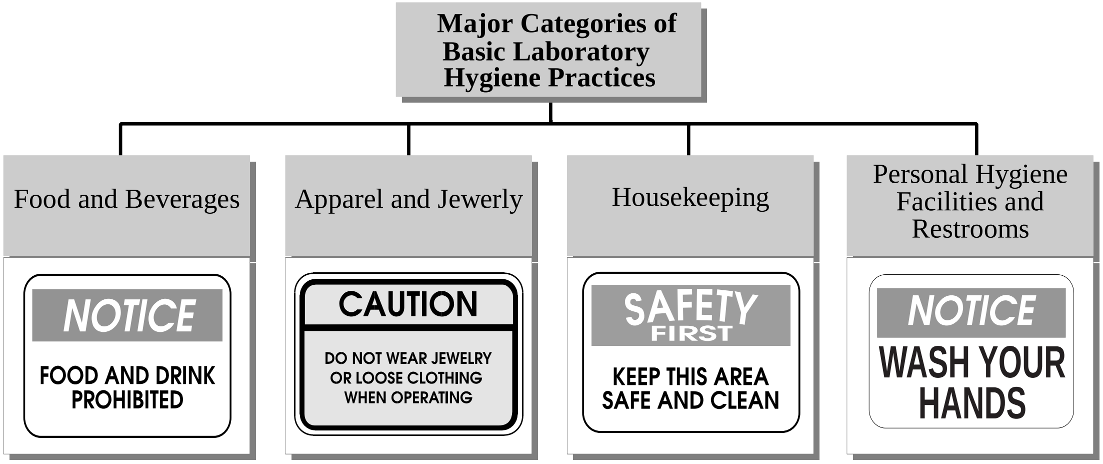
For additional information on laboratory clothing, refer to Chapter E2 of this manual.

The jewelry being worn must be limited to small items with little chance of projection into operations. Rings must be removed during materials handling, mechanical work, or operations involving contact with chemicals or solvents. Hands should be washed thoroughly after any contact with a chemical or solvent, prior to replacing rings. Watches become magnetized when near large magnets such as those found in nuclear magnetic resonance (NMR) machines, mass spectrometers, etc. They must then be demagnetized to keep reliable time.

2.3 Housekeeping

Laboratory supervisors and employees are responsible for keeping their work areas neat and orderly. All aisles, walkways, halls, and exits must be kept clear, dry, and free of obstructions and hazards, such as bottles, boxes, and open drawers or cabinets, to allow safe passage.

Figure F2-1: Major Categories of Basic Laboratory Hygiene Practices



Step stools should be kept out of passageways when not in use. Equipment should be placed back from the edge of tables and benches, or be placed in such a position to minimize the possibility of it striking laboratory employees or obstructing their exit. Also, emergency equipment (e.g., eyewash stations, safety showers, and fire extinguishers, etc.) should be readily accessible and not obstructed by equipment.

The following practices are recommended to provide safe storage, use, and maintenance of laboratory equipment:

- Establish a definite storage place for each item; return all apparatus, equipment, or reagents to their proper location immediately after use. Carefully choose the storage location for hazardous materials.
- Make certain that connecting lines such as flexible tubing and power cords are not longer than necessary; remove defective equipment (including equipment with frayed electrical cords) from service until it is repaired.
- Dispose of cracked or broken glassware by placing it in a separate receptacle. Under no circumstances should damaged glassware be used.
- Properly ground equipment in which a buildup of static electricity may be expected (e.g., when pouring solvents or transferring dry powder).
- Wash nondisposable personal protective equipment (PPE) frequently to reduce the possibility of irritation or contamination.

2.4 Personal Hygiene Facilities and Practices

Handwashing with lukewarm water and antimicrobial soap (not bar soap) must be performed immediately following removal of gloves and other PPE. When handwashing facilities are not available, antiseptic hand cleansers or antiseptic towelettes must be used until the individual is able to wash his or her hands.

3.0 General Safe Laboratory Work Practices

Policies and procedures are in place at EPA laboratories to promote employee safety. Operational and handling techniques to be employed in the laboratory are described in the following sections and the major categories covered are summarized in Figure F2-2.

3.1 Employee Conduct

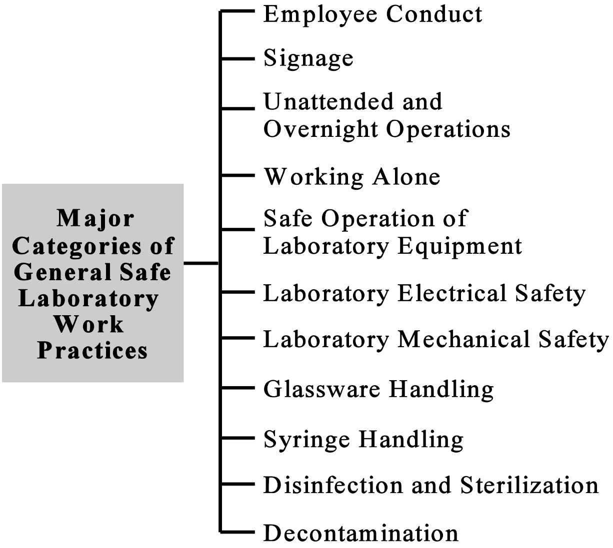
The proper conduct of employees in EPA laboratories is critical to promoting a safe working environment. Safe laboratory work requires an alert, clear mind. Any person who is under the influence of alcohol or drugs is a hazard to himself and fellow employees.

NOTICE

THE USE OF ALCOHOL
ON THE PREMISES
WILL MEAN IMMEDIATE
DISMISSAL

NO RUNNING

Figure F2-2: Major Categories of General Safe Laboratory Work Practices



Running in laboratory buildings is prohibited unless life is threatened by an extreme hazard such as sudden release of toxic or flammable gases. A fast walk will suffice for an escape or for a response to an emergency.

3.2 Signage

Prominent signs and labels of the following types should be posted throughout the laboratory:

- Identity labels showing contents of containers and associated hazards

- Location signs for safety showers, eyewash stations, other safety and first-aid equipment, exits, and areas where food and beverage consumption and storage are permitted

- Warnings at areas or equipment where special or unusual hazards exist

- Emergency telephone numbers and evacuation routes

Figure F2-3 presents some examples of these signs.

There are regulations and guidelines that require specific signs and labels for specific hazards. These specific requirements can be found in chapters of this manual that pertain to specific hazards. Examples include:

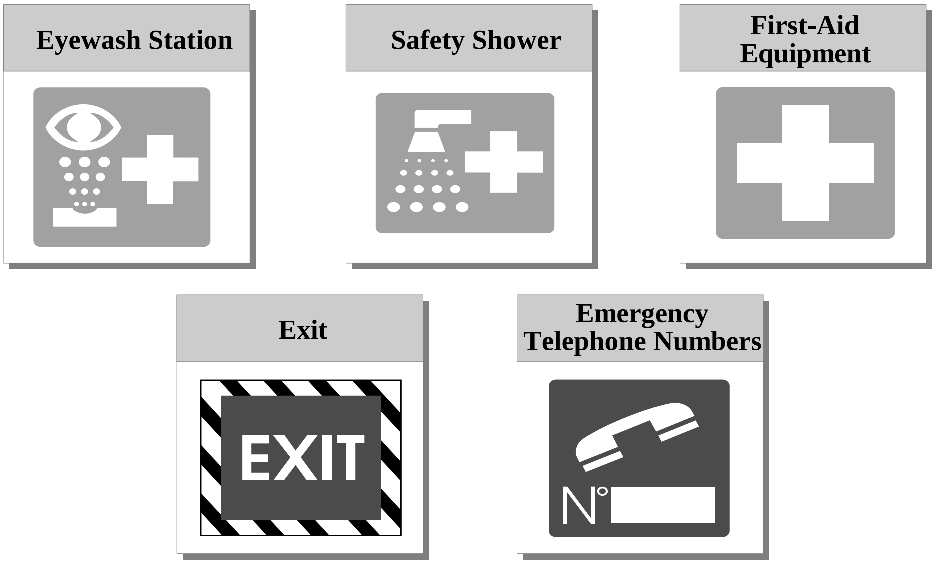
C6	Radiation Safety
C7	Biosafety
E4	Safety Showers and Eyewash Stations
F3	Work Practice Controls for Chemicals
G3	Emergency Systems and Equipment

3.3 Unattended and Overnight Operations

At EPA laboratories there may be unattended and overnight operations such as extractions, stirred reactions, and refluxing solvents. Special precautions should be taken if any of the following conditions are present:

- Room temperature and/or pressure changes.
- Flammable, hazardous, or reactive chemicals are used.
- Equipment depends on utilities for continued safe operation.

Figure F2-3: Examples of Signage Posted in a Laboratory



- Radiant energy (emitted or transmitted) is used.

Special precautions to be taken under these conditions include the use of containment, automatic shutdown, and appropriate location and warnings.

If working at night, on weekends or holidays, laboratory employees may be alone and without immediate assistance in an emergency. In these situations, work performed should be limited to low-hazard procedures (e.g., office work, gravimetric weighing), avoiding potentially hazardous operations such as:

- Using flammable solvents
- Handling corrosive or toxic chemicals
- Manipulating large glass apparatus
- Working with experimental electrical or radioactive systems

If potentially hazardous procedures must be performed during off-hours, accompaniment should be arranged.

An additional consideration during overnight operations is that the ventilation in some EPA laboratory buildings may not be operating during off-hours (6 p.m. to 6 a.m.). If work must be performed during this period, the building manager should be contacted to maintain the ventilation in the area.

3.3.1 Containment

The following containment precautions can be taken to reduce risk during unattended and overnight operations:

- Provide adequate exhaust ventilation for chemical vapors.

- Place a metal or plastic pan under breakable glassware to contain spills.

- Operate heating elements through a variable transformer set at the lowest voltage that will maintain the desired temperature.

- Wire or clamp all water tubing to prevent release of water in case of a pressure surge.

3.3.2 Automatic Shutdown

Automatic shutdown can be accomplished by the use of a circuit breaker to stop the current in case of a malfunction or electrical short. If possible, a monitoring device should be used to stop the operation if conditions deviate from safe limits.

3.3.3 Location

The location of the experimental apparatus and other laboratory operations can reduce risk. The following are precautions that can be taken:

- Place the apparatus where it does not interfere with other operations.
- Keep all operations visible from the doorway, if possible.
- Post the names and telephone numbers of those familiar with the operation on the laboratory door.
- Do not lock the laboratory door overnight.

3.3.4 Warnings

The following precautions can be an effective means of reducing risk:

- Post signs to warn laboratory employees and staff of possible dangers.

- Use buzzers or other warnings to alert laboratory employees and staff of malfunctions if hazardous materials or hazardous operations are involved.

In cases where an unattended overnight operation is planned, the experimental set-up should be reviewed. If the hazard level is acceptable, then an authorization for a specified time can be issued.

3.4 Working Alone

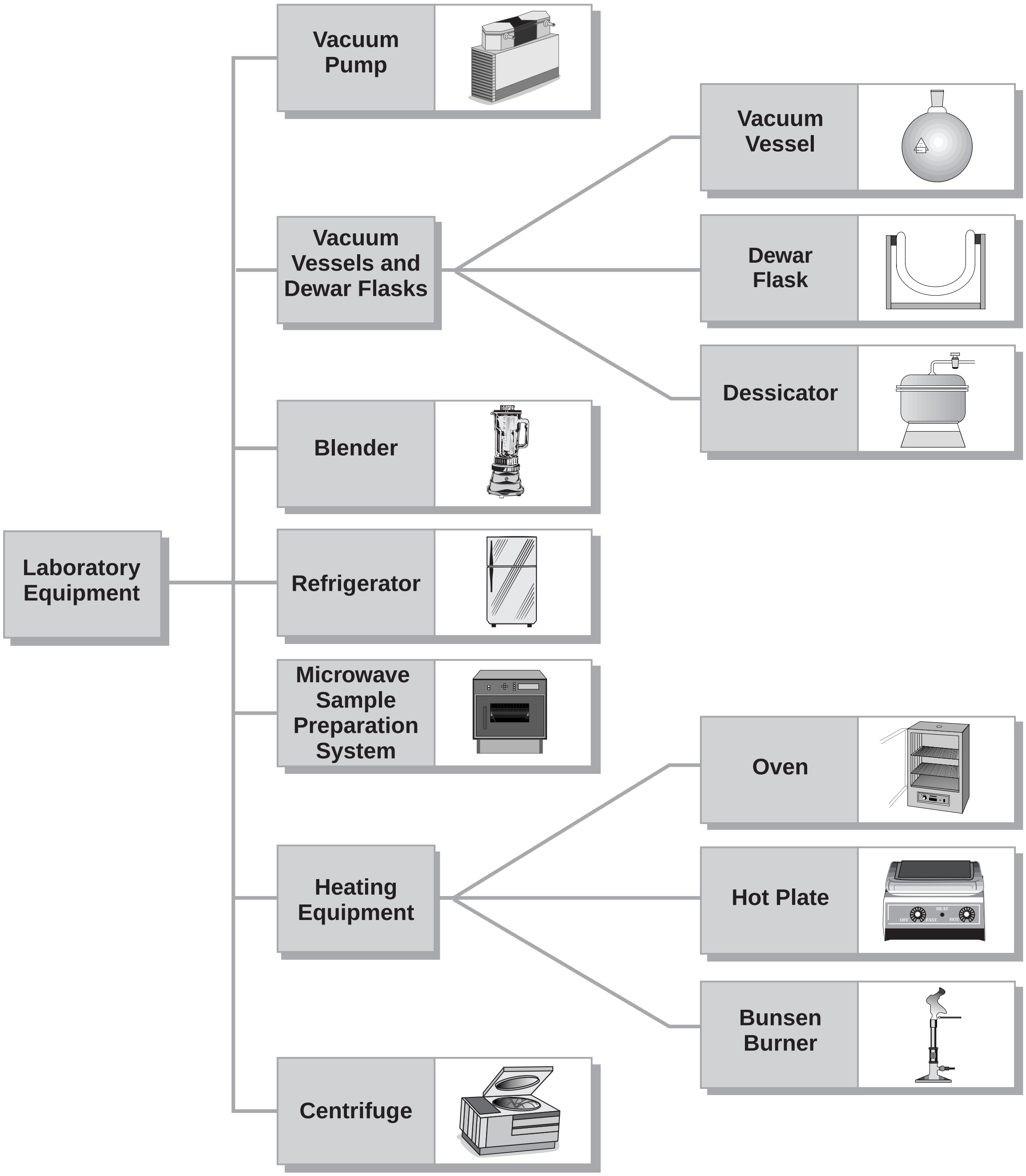
When any individual is performing work out of audio or visual range of another individual for more than a few minutes at a time, they are considered to be working alone. As a general policy, if at all possible, no one should work alone or under conditions where emergency aid is not available.

The availability of emergency aid, the degree of training, the type of the emergency aid, and the means of summoning the help depend on the nature of the hazard and the degree of exposure to the hazard. Most work assignments can be set up with the appropriate emergency aid available. Whenever there is a doubt concerning a work assignment and this philosophy, the principle investigator and Safety, Health and Environmental Management Program (SHEMP) Manager should review, and jointly define, the work assignment and the emergency aid compatible with the work assignment.

3.5 Safe Operation of Laboratory Equipment

The safe operation of laboratory equipment is an important part of work practice controls. Some common examples of laboratory equipment are discussed below and shown in Figure F2-4.

Figure F2-4: Examples of Laboratory Equipment



3.5.1 Vacuum Pumps

Every vacuum pump used in the laboratory must have a belt guard in place whenever it is operating. Its service cord and switch must be free of observable defects. A trap should be used 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 dilution of the pump oil. A pan under the pump is useful to catch any dripping oil.

If the pump is used for vacuum distillation or filtration of organic liquids, the discharge should be directed to an operating hood or other exhaust system. Discharge into an enclosed space, such as a laboratory cabinet, can lead to an explosive release of vapors.

If the pump is used in an area where flammable gas, vapors, or dust (which, when present in large enough quantities, can form explosive mixtures in air) are present, the motor, cord, plug, and all other electrical parts must be explosion-proof.

3.5.2 Vacuum Vessels and Dewar Flasks

Glassware items used for vacuum distillation or other uses at reduced pressure must be properly chosen for their ability to withstand the external pressure of the atmosphere (e.g., round-bottom vessels or other types, such as Erlenmeyer filtration flasks, that are specially designed for vacuum use). Each vessel must be carefully inspected before each use for defects such as scratches, star cracks, or etching marks. At reduced pressure, glass vessels can collapse violently, either spontaneously (if cracked or in some other way weakened), or from an accidental impact. All vacuum equipment is subject to failure by implosion. The atmospheric pressure propels pieces inward and they then continue outward with considerable force. For this reason, all vacuum operations must be carried out behind a table shield or lowered hood sash.

Dewar Flasks

Dewar flasks should not be filled to more than 80 percent of capacity. This limit is a precaution against possible thermal expansion of the contents and bursting of the vessel by hydrostatic pressure.

Pouring cold liquid onto the edge of a Dewar flask may cause the flask to break and implode. Liquid nitrogen must never be poured out of a Dewar flask. Dewars can collapse as a result of thermal shock or a very slight scratch by a stirring rod. Glass Dewar flasks have a vacuum between the walls, and, upon failure, can propel glass into the eyes. For this reason, all glass Dewars should be wrapped from top to bottom with cloth tape, such as electrician’s friction tape, or, if transparency is needed, mylar tape. They can also be enclosed in a wooden or metal container, to reduce the hazard of flying glass in case of collapse. Stainless steel vacuum containers are also safe, as received. However, these containers must be properly vented. Section 3.8.5 provides more information on the use of Dewar vessels for cryogenic materials.

Glass Desiccator

Glass desiccators are often subjected to partial vacuum caused by the cooling of their contents. They have inherent strains due to glass thickness and the relatively flat top and bottom surfaces. A desiccator guard made of perforated metal is available and is strongly recommended. An alternative is to use a molded spherical plastic desiccator with high tensile strength.

3.5.3 Blenders

Household-type blenders used in the laboratory are a common cause of solvent fires. The motors of such blenders have constantly sparking brushes that can readily ignite any released vapors or liquid splashed out of the cup. Consequently, solvents having a flash point below 100°F (37.8°C) must never be used in these blenders.

A catch pan under a blender is useful for limiting spills of liquids or solids from the cup. If a fire should occur, its extent will be limited and extinguishing it should be easy. An explosion-proof blender is available in a one-liter size. However, it has a slow motor and is very expensive. As such, the type of blender used should be selected based on the intended use and associated hazards.

3.5.4 Refrigerators

Food must never be placed in a refrigerator or freezer used for chemicals or biological agents. If food is found in a chemical refrigerator, it must be removed and discarded. Refrigerators designated for food storage must not be located in laboratories.

All refrigerators must be marked either:

NOTICE
FOOD ONLY

NOTICE
CHEMICAL ONLY

No flammable liquids (i.e., flash point below 100°F [37.8°C]) may be placed in an ordinary domestic-type refrigerator or freezer. Flammable liquids can vaporize, forming flammable mixtures that explode when the thermostat sparks. Likewise, carcasses containing ether or materials soaked in alcohol are not permitted in domestic-type refrigerators or freezers. Refrigerators and freezers must have signs on the doors specifying their use.

NOTICE
NO FLAMMABLE LIQUIDS

When refrigeration is needed for flammable materials, there are two options. If the refrigerator is in a room in which hydrocarbon gases or vapors may be present, an explosion-proof refrigerator should be specified. If the appliance will contain flammable materials but be in a room free of vapors or gas, an explosion-safe unit can be purchased at lesser cost. Either type, however, must include an appropriate warning sign:

NOTICE
EXPLOSION-PROOF

NOTICE
EXPLOSION-SAFE

If materials stored in a refrigerator are sensitive to elevated temperatures, a battery-powered alarm should be installed to signal an elevated temperature, that may occur due to power loss.

3.5.5 Microwave Sample Preparation Systems

Microwave sample preparation systems are being used to drastically reduce the time required to perform digestion of both inorganic and organic samples. The safety devices built into the units vary by the manufacturer and the age of the unit. In addition, safety measures taken by researchers depend on the type of sample digestion being performed (e.g., inorganic or organic). Following are specific recommendations for digestion of inorganic and organic samples in a microwave sample preparation system.

Inorganic Sample Digestion

A mixture of concentrated acids under high temperature and pressure is used during the inorganic sample digestion process.

Organic Sample Digestion

A mixture of concentrated acids and solvents under high temperature and pressure is used during the organic sample digestion process.

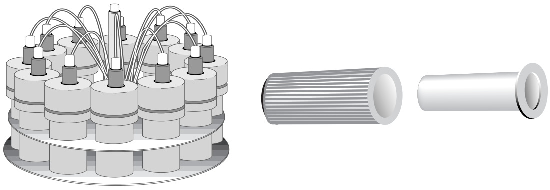
The microwave systems that are designed to use potentially flammable solvents should be equipped with additional safety devices such as: vibration sensors, solvent sensors, and a button-latch door.

In both types of sample digestions, to protect against acid burns if a vessel should rupture, laboratory employees should be equipped with PPE such as an apron, face shield and goggles, and neoprene gloves (gauntlet length). When the microwave door is opened, the pressure inside the sample vessels should be monitored to ensure that it has dropped to 20 pounds per square inch.

A negative pressure should be created inside the microwave cavity by drawing air from the back of the microwave system into the laboratory ventilation system. This will prevent fumes from entering the laboratory employee’s breathing zone.

Sample Vessels and Caps

During the microwave digestion process, only one of the sample vessels is monitored for pressure through the attachment of a sensor. The vessel selected for attachment to the sensor should be representative in contents to the other vessels.



Both sample vessels made of polyether emid and sample caps with a Teflon™ coating have been shown to deteriorate over time. Sample vessels made of polyether emid can show bowing of the sides of the vessel. Vessel caps can show bubbling, blistering, and peeling of the Teflon™ coating on the underside of the caps. This could be due to acid fumes escaping during the uncapping of the vessels or processing of the caps through the glass wash using an acid solution, which is typically not required for the caps of the vessels.

Manufacturers recommend that each vessel be used no more than 100 times. As such, each vessel should be given a serial number and the number of uses tracked with a log sheet. However, if any signs of deterioration are seen prior to the 100 uses, the vessel should be taken out of use and properly disposed of.

3.5.6 Heating Equipment

A variety of heating equipment is used in the laboratory during experimental procedures. Ovens, hot plates, bunsen burners and other heating equipment should be used with caution to avoid burns and ignition of combustible materials. Ovens should have reliable and well-maintained thermostatic controls. Hot plates should have an on-off switch, enclosed wiring, and be designated for laboratory use. Bunsen burners, as well as any equipment with an open flame, should be used with extreme care to prevent ignition of combustible or flammable materials in the laboratory.

3.5.7 Centrifuges

Centrifuges used in a laboratory can be one of two models: benchtop and floor. EPA laboratory staff who use centrifuges should be certain that the centrifuge is securely anchored (e.g., suction cups or wheel brakes) and balanced each time it is used. In addition, they should be interlocked so that the rotor shuts off if the top is opened during motion.

3.6 Laboratory Electrical Safety

The following sections provide an overview of the important aspects of electrical safety applicable to the laboratory. Refer to SHEM Guide 30 “Electrical Safety” for detailed information on electrical safety.

A 115-volt, 60-hertz current of only 1/10th of an ampere (100 milliamperes) may be fatal if it passes through vital organs. The fatality usually occurs because of ventricular fibrillation, which interrupts normal heart action. Ventricular fibrillation can occur as long as two weeks after the initial shock. Therefore, any time even the slightest electrical shock is received, it is recommended that an electrocardiogram (EKG) be performed to detect any heartbeat irregularities. If the shock occurs on the job at an EPA laboratory, the SHEMP Manager must be contacted immediately.

To protect laboratory employees from electrical shock during the servicing or maintenance of machines and equipment, the U.S. Occupational Safety and Health Administration (OSHA) has promulgated lockout/tagout regulations in 29 CFR 1910.147. These regulations establish minimum performance requirements to control hazards such as the unexpected start-up of machinery or equipment, or the release of stored energy that could cause injury to employees. SHEM Guide 34, “Lockout/Tagout,” provides additional information on shock protection.

Most electrical work at EPA laboratories requires a permit issued by the appropriate local and state officials to ensure that the work meets local and state building codes. To protect EPA employees and property, electrical work is restricted as follows:

- Work on electrical circuits and equipment, power lines, and power outlets is restricted to qualified electricians, except where research is being done on instrumentation and other electrical or electronic circuits.
- All electrical installations shall be in accordance with the National Electrical Code and must be appropriate for the kinds of materials found in the area. All conduits, outlets, and equipment that could involve physical contact must be grounded.
- All 115-volt outlets and plugs in EPA laboratories shall be three-wire grounded. Exceptions are made only for equipment that cannot be grounded, such as clocks.

If there is a chance that an accidental spill of a liquid may increase the conductivity of floors, benches, etc., use of a ground-fault circuit interrupter (GFCI) should be considered. Plugging equipment into this device will protect employees from electrocution by leakage to ground. The National Fire Protection Association (NFPA) has specific guidelines for when GFCIs should be used.

3.6.1 Extension Cords

Extension cords waste energy, are subject to damage, and constitute tripping hazards. The use of extension cords in place of permanent wiring is not allowed. The use of extension cords should be limited to temporary needs such as hooking up portable equipment and power tools. OSHA has interpreted temporary to mean less than eight hours. If the cord from a permanent piece of equipment, such as a refrigerator or chromatograph, does not reach an outlet, the equipment should be relocated or a new outlet installed within accessible range.

Multiple-outlet devices attached to a length of wire are commonly used in laboratories. These devices must have circuit interrupters. Unfortunately, there is a temptation to overload these devices without considering that they plug into only one of the outlets on a circuit. Where additional outlets are needed, they should be installed, even if additional branch circuits must be run.

Furniture and equipment in offices and laboratories should be arranged so that access to electrical panels is clear. Electrical panel doors and locks must be maintained in working order. A three-foot access aisle to electrical supplies and transformers must be provided.

3.6.2 Static Electricity

Laboratory employees must guard against the dangers of accumulation of static electricity, that may generate sparks.

Nonconductive surfaces moving past each other generate static electricity. Therefore, even certain forms of safety clothing, such as plastic shoe coverings, may produce static electricity. It is advisable to use grounded conducting surfaces, and conducting materials, in cases where there is a danger that explosive mixtures of gases may be present.

3.7 Laboratory Mechanical Safety

In the normal course of EPA laboratory work, nothing more than standard PPE will be needed as a safeguard. However, some types of operating equipment should have guards provided to protect laboratory employees and passersby.

It is dangerous to leave a guard off of any piece of machinery or equipment. If a guard is removed from a machine for maintenance or any other reason, the machine must be made inoperative until the guard has been replaced.

Whenever work takes employees near equipment in any of the categories covered in this section, and shown in Figure F2-5, proper guards must be in place.

3.7.1 Equipment Guarding

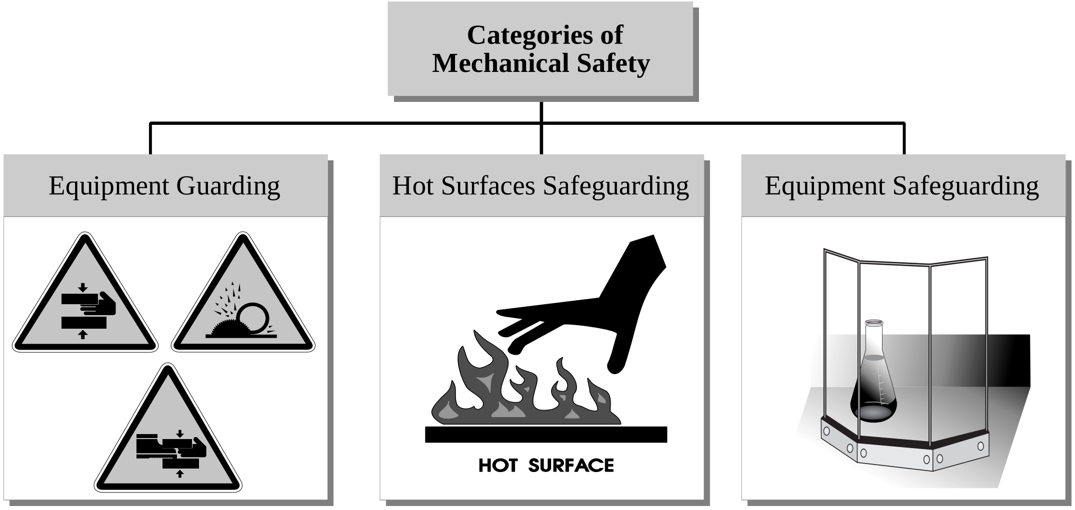
Rotating equipment, gears, belt drives, in-running rolls, and other power transmission machinery must be guarded so that employees are not injured by accidental contact. Wherever moving parts can catch hands or clothing, a guard should be installed over the moving parts.

Guards of ample strength should be permanently anchored around any rotating units, such as fan blades and grinding wheels, that could break or separate.

Every power-driven machining tool (e.g., saw, shaper, drill, grinder, etc.) should have its own hinged guard, which should ride over the work but protect the hands against accidental contact; or a point-of-operation guard. Examples of moving parts that require guarding include:

- Shaft collars or other rotating members with protruding parts
- Where belts engage their pulleys
- Where chains engage their sprockets
- Where gears mesh

Figure F2-5: Categories of Mechanical Safety



Whenever oil or other fluids may be thrown off from a rotating unit or squirted out of any vessel or machinery, a shield should be placed to prevent the debris from striking the floor and rendering it slippery, or from striking employees in the vicinity.

If machinery is commercially constructed with point-of-operation guards, they should not be removed. If an employee finds a guard off, they should not operate the machine until the guard is replaced. If an employee takes a guard off for any purpose, it should be replaced before they leave the equipment or area. Remove guards only when equipment has been properly deenergized, and when it needs to be repaired. Do not remove guards simply for convenient operation.

If a laboratory setup is being designed or constructed involving power transmission or mechanical work, all nip points should be guarded. If new mechanical equipment with exposed moving parts is requested, it should be determined whether it is, or can be, factory-equipped with guards. If a vacuum pump or other motor-driven equipment is ordered, be sure a belt guard is part of the order.

Some equipment, such as dynamometer rolls and rubber mills, is extremely hard to properly guard. Sawhorses or ropes can be used to enclose an area. Limit switches can help safeguard rolls and mills, but proper operator safety training is essential to prevent serious accidents. SHEM Guide 35, “Machine Guarding and the Use of Hand Tools,” contains additional information on machine guarding.

3.7.2 Hot Surfaces Safeguarding

Touching a surface that is 150°F (66°C) may not cause a burn, but it will cause a reflexive jerk of the hand away which may cause it to strike something else. Because of the possibility of personal injury or damage to delicate equipment, any surface at 150°F (66°C) or above should be guarded against personal contact.

Above 200°F (93°C), the added hazard of ignition of flammable materials exists. Any surfaces reaching this temperature should be guarded, not only against personal contact, but also against possible exposure to chemical drips or spills.

In many cases, an adequate layer of insulation will serve to prevent contact with a hot surface. Steam lines and hot oil lines, for example, must be insulated whenever physical contact is possible.

3.7.3 Equipment Safeguarding

Reactors, stills, or other equipment operating at pressures or temperatures significantly above or below ambient levels, or containing hazardous materials, may require shielding to protect laboratory employees and property in the vicinity.

A self-supporting plastic shield is the minimum for bench-top work. It is required for laboratory glassware under pressure or vacuum, and for glassware containing:

- Corrosive chemicals
- Reactive chemicals
- Chemicals that can be absorbed through the skin
- Unknown reactions
- Exothermic reactions

The SHEMP Manager may prescribe a steel barricade surrounding any metal equipment that:

- Contains undefined reactions
- Contains exothermic reactions
- Operates at very high pressure

Whenever operating pressure will be above 15 pounds per square inch, the equipment must be inspected and approved in accordance with the American Society of Mechanical Engineers (ASME) Pressure Vessel Code.

Any equipment that contains radioactive materials or that can produce ionizing radiation must be installed and operated in accordance with directions of the radiation safety officer. Refer to Chapter C6 of this manual for more information on radiation safety.

3.8 Glassware Handling

Glass is strong during compression and tension, but weak under bending stress. Scratches, severe temperature changes, seals between different types of glass or poor annealing, can cause glass to break.

A large percentage of laboratory injuries are due to glass cuts. Most cuts cause only superficial injury but some glass cuts may puncture blood vessels or sever tendons.

The following are some precautions for use of glass under various conditions.

3.8.1 General Glassware Precautions

Use only the right size and type of glassware for any given procedure and be sure it is in good condition before use. Avoid chipped, cracked, or scratched glass. Any broken pieces of glassware should be discarded in the proper container unless they are sufficiently valuable to warrant repair.

Inspect all glassware to be used under pressure or vacuum. Round-bottom flasks used for vacuum distillations are a special problem. This type of glassware frequently gets scratched or cracked in handling, even by letting one piece rub against another. Examine and anneal any flasks or other glassware to be used under pressure or vacuum.

3.8.2 Glass Tubing

Pushing a glass tube into a rubber stopper is one of the most common, yet most hazardous, laboratory operations. Be sure the tubing end has been fire-polished and both the tubing and stopper are lubricated. If heavy leather gloves are not readily available, wrap several layers of cloth around the tubing and the stopper. Use the same precautions for similar procedures with any piece of glass tubing.

3.8.3 Glassware Under Pressure

Most laboratory glassware is dependable only near atmospheric pressure. Certain types of glass bottles or other glass vessels have been used to carry out chemical reactions at moderate pressures.

Minimum precautions to be followed when using glassware under pressure include the following:

- Do not exceed the manufacturer’s recommendation for maximum working pressure and temperature.

- Use only reactors designed to be safe for the reactions occurring.

- Discard any bottles subjected to hard impacts or that have observable scratches or other defects on the surface.

- Ensure the reactor, when under pressure, is inside a perforated metal sleeve or similar protection.

- Use supplementary shielding, such as a plastic half-cylinder, as additional protection.

- Keep glassware under pressure behind a shield or within a suitable guard. Wear heavy gloves when handling or reaching around any glass apparatus that is under pressure.

Glass vials sealed with a flame are sometimes used as containers for chemicals or samples. Heavy-walled glass tubes are sometimes sealed and used as reaction vessels. Excessive pressure, thermal shock, mechanical shock, or faulty glass in one of these vials can result in a violent rupture.

Use extreme care to prevent such breakage. Table shields or other devices must be used to protect the laboratory employee and others from the chemical contents of the tube, glass fragments, and heating bath liquid. Temperature should be raised and lowered slowly, and a cushion should be provided for protection against mechanical shocks. Presented below are safe temperatures for glass.

Soft glass (lead alkali)	≤ 230°F (110°C)
Borosilicate glass (Pyrex™, kimax)	≤ 700°F (370°C)
Quartz (Vycor™)	≤ 1690°F (900°C)

3.8.4 Glassware Under Vacuum

Vacuum or pressure operations need approval from the SHEMP Manager if the glassware to be used has more than a two-liter capacity.

An implosion can scatter sharp-edged glass fragments just as violently as an explosion of a vessel. Ensure that any glass equipment under vacuum pressure is shielded or guarded as safely as pressurized equipment.

Certain types of glass equipment, such as heavy-walled filtration flasks, are made for use under vacuum. Vacuum distillations in glass should be performed only in round-bottom flasks.

3.8.5 Glass Dewar Flasks and Vessels

Many double-walled vessels are used throughout EPA laboratories to transport or handle cryogenic materials. Because the space between the two walls is a vacuum, a possibility always exists for implosion. A crack in the inner wall may allow the cryogenic material to leak in between the walls, causing a sudden expansion of gas and possibly an explosion of the Dewar.

Many Dewars are encased in a metal sheath to prevent physical shock. Smaller Dewars are often unprotected and must be wrapped in friction tape or other secure binding and coated with a special plastic dip, or encased in metal. Even when protected, Dewars must be handled carefully to prevent breakage.

3.9 Syringe Handling

Clinical-type hypodermic syringes are frequently used to:
- Measure and transfer small quantities of fluid components
- Administer injections to animals
- Inject materials into instruments
- Withdraw liquids from vials or bottles kept closed by rubber diaphragms or septa

Syringes are available in various capacities. The needles, which range in length from ¼ inch to over 6 inches, are identified by gauge numbers. Techniques for handling syringes and needles vary with size and the liquid being handled, but the following general rules always apply:

- Place an alcohol-moistened pledget around the stopper and needle when a syringe and needle is removed from a rubber-stoppered vaccine bottle containing infectious material.

- Use only needle-locking hypodermic syringes.

- Always choose the shortest needle that will serve the purpose, as shorter needles are less likely to bend or break.

- Vertically expel excess fluid and bubbles from syringes into disposable cotton pledgets. Moisten cotton pledgets with a disinfectant for use with infectious materials. Do not hold a needle vertically due to the potential release of airborne contaminants.

- Reusable sharps, such as large-bore needles and scalpels, must be stored in a manner in which sharp ends are not exposed.

- Reusable sharps may be stored in shallow pans as long as employees use mechanical devices to retrieve them from the pan.

3.9.1 Syringe Capacity

Never fill a syringe above its graduated capacity. The extra length of the barrel is necessary to guide the plunger during ejection of the fluid from the syringe. Without this length of guide, the plunger may jam and break the syringe barrel. The syringe should not be more than half-filled with a slurry containing solid particles.

3.9.2 Glass Syringes Under Pressure

The working limits for glass syringes vary with diameter. Excessive force on the plunger may cause the barrel to burst, releasing toxic or corrosive material.

When syringes are used to inject materials into instruments such as gas chromatographs, stand to the side of the injection port, not directly in front of it. Keep your thumb on the end of the plunger throughout the injection procedure. When the syringe first enters the injection port, the difference in temperature (especially for very volatile compounds) tends to force the plunger out of the syringe.

3.9.3 Temperature Extremes

Many syringes are made of soft glass, so they may not withstand thermal shock.

They should not be heated above 250°F (120°C), nor should they be chilled or heated suddenly.

3.9.4 Cleaning of Syringes

Wash a syringe immediately after each use. Flush appropriate solvent such as naphtha, alcohol, or acetone through the barrel and over the plunger. Do not use any abrasive material, either dry or in liquid suspension. The soft glass may be scratched, allowing the next fluid used to squirt out the plunger end.

3.9.5 Disposal of Needles

When disposing of needles, place them in puncture-resistant, closeable sharps containers. Disposable needles must be placed directly into designated sharps containers after use without recapping, shearing, clipping, or breaking the tip. Always locate sharps containers in the work area. In cases where it is absolutely necessary to recap needles (e.g., remote location where no sharps containers are nearby), use some type of device that protects the hand, or use the one-handed recapping method.

If the sharp item was used with, or exposed to, human blood or other potentially infectious materials, the sharps container must be color-coded red and labeled with the universal biohazard symbol. Refer to Chapter C7 of this manual for more information on biosafety.

All waste needles and syringes must be placed in puncture-resistant, closeable primary containers. Never place needles in trash cans, glass bottles, or any container that is not puncture-resistant. Refer to Chapter C14 of this manual for more information on sharps disposal.

3.10 Disinfection and Sterilization

Disinfection is an important procedure to follow when handling any biohazardous or toxic agents. Work surfaces used for biohazardous agents must be disinfected using an approved disinfectant after completion of the work, upon visible contamination, after spills, or at the end of the work day.

Inspect all bins, pails, cans, and similar receptacles that have been in contact with biohazardous agents. Check for contamination and disinfect regularly or upon visible contamination.

3.10.1 Heat Sterilization

The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Moist heat, or steam, at 250°F (120°C) under pressure (15 pounds per square inch) in the autoclave is the most convenient method of rapidly achieving sterility. However, sterility is not guaranteed by the machine merely reaching this temperature, since many variables can influence the effectiveness of the autoclave.

Several factors affect the steam sterilization process including load size, distribution and compaction, altitude above sea level, and heat penetration. The investigator or personnel responsible for sterilization may have to determine the appropriate time at standard autoclave temperature and pressure for certain loads of biohazardous materials.

Therefore, the autoclave should be monitored to ensure that the procedure used results in full sterilization. Two methods of monitoring exist, chemical and biological. Chemical, although adequate for routine daily monitoring, is not considered an acceptable method of testing. Biological monitoring is done with spores, usually *Bacillus stearothermophilus*, which can survive 250°F (120°C) for 13 minutes. These microorganisms are more resistant to temperature than most and thus provide an adequate safety margin when validating sterilization procedures.

Laboratory employees should be cautious because steam under pressure can be a source of scalding jets if the equipment for its application is mishandled. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too promptly after treatment. This can cause a sudden and violent boiling of the contents from containers that can splash scalding liquids onto personnel handling the containers.

Dry heat at 160° to 170°C for periods of two to four hours is suitable for destruction of viable agents on impermeable nonorganic material such as glass. However, it is not reliable for even thin layers of organic or inorganic material which can act as insulation. In this case, incineration kills microorganisms and serves as an efficient means for disposal.

3.10.2 Radiation Sterilization

Ionizing radiation will destroy microorganisms and has been used for sterilization, however, it is not a practical tool for laboratory use. Nonionizing radiation such as ultraviolet radiation (UV), is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. The nonionizing radiation is especially useful for the destruction of airborne microorganisms, the inactivation of microorganisms on exposed surfaces, or the treatment of products of unstable composition that cannot be treated by conventional methods.

The usefulness of UV radiation as a sanitizer is limited by its low penetrating power. Information is not available regarding the effectiveness of UV radiation for inactivating microorganisms containing recombinant DNA (rDNA) molecules, but it is highly unlikely that increased resistance to UV radiation is imparted to a cell by the insertion of rDNA. UV radiation is primarily useful in air locks, animal holding areas, ventilated cabinets and in laboratory rooms during unoccupied periods to reduce the levels of viable airborne microorganisms and to maintain good air hygiene.

The use of UV radiation carries the danger of burns to the cornea and the skin if exposed for even a short time. Proper shielding should be maintained where irradiation treatment is used when personnel and laboratory animals are present. Guard against reflecting surfaces (e.g., polished stainless steel) occurring in line with the light source. In areas irradiated without shielding on special occasions or during off-duty hours, post the area with warning signs to prevent unscheduled entry of personnel. Refer to Chapter C6 of this manual for more information on radiation safety.

3.10.3 Liquid Disinfectants

In general, liquid disinfectants are most practical for use in surface treatment and, at sufficient concentration, as sterilants of liquid waste for final disposal in sanitary sewerage systems. Liquid disinfectants are effective in the test tube but may fail in a practical situation. Failures often occur because proper consideration was not given to:

- Temperature
- Time of contact
- pH
- Concentration
- The presence and state of dispersion, penetrability and reactivity
- The presence of organic material at the site of application

Small variations in these factors may make large differences in effectiveness of disinfection. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid disinfectants when the end result must be sterility.

Selection

There are many liquid disinfectants available under a wide variety of trade names. In general, these can be categorized as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones alcohols, and amines. Unfortunately, the more active disinfectants often possess undesirable characteristics, such as corrosive properties. No type of liquid disinfectant is equally useful or effective under all conditions.

Laboratory personnel should be familiar with the various disinfectants that will effectively kill the biohazardous agents being used. The following information is provided to assist in the selection of appropriate disinfectants such as: alcohols, chlorine compounds, iodophors, phenolic compounds, quarternary ammonium compounds, and formaldehyde solutions.

Alcohols

Ethyl and isopropyl alcohol are good disinfectants for the vegetative forms of bacteria and lipoviruses.

When used in a dilution of 70 to 95 percent, ethyl alcohol effectively inactivates vegetative bacteria and lipoviruses; has variable results with inactivating non-lipoviruses and is ineffective with inactivating bacterial spores.

Some of its other characteristics include: flammability, eye irritation, and toxicity (threshold limit value [TLV] of 1000 parts per million [ppm]).

Isopropyl alcohol has the same effectiveness at inactivating biohazardous agents as ethyl alcohol. However, its toxicity is greater (TLV of 400 ppm).

Chlorine Compounds

The germicidal effect of chlorine compounds is dependent upon the release of hypochlorous acid and is therefore dependent upon the available chlorine. A contact time of 10 to 30 minutes should be allowed.

A use-dilution of 500 ppm available chlorine is recommended for vegetative bacteria and most viruses. Chlorine solutions that are neutral or slightly acidic, and with a concentration of approximately 2,500 ppm chlorine, are needed for effectiveness against bacterial spores. Undiluted common household bleach is alkaline with a pH of 8 or greater. Household bleach typically contains 5.25 percent sodium hypochlorite for 52,500 ppm of available chlorine.

Chlorine compounds are corrosive to metals; leave a residue; irritate the skin, eyes, and respiratory tract, and are toxic. They are also rapidly inactivated by organic matter. While chlorine compounds are not generally recommended for routine use, undiluted household bleach is frequently used with biological spills.

Iodophors
The germicidal effect of iodophors is dependent on the free iodine released from the compound in which it is contained. A contact time of 10 to 30 minutes should be allowed.

Use-dilutions can range from 25 to 1,600 ppm of available iodine, whereas solutions containing 75 to 150 ppm are generally recommended for use.

Iodophors inactivate vegetative bacteria, fungi, and viruses but have poor activity against bacterial spores.

Although iodophors are less harmful to man than chlorine compounds, they can irritate the skin and eyes. Iodophors are corrosive (less than chlorine), they leave a residue, and may stain. However, iodophor stains can be readily removed with solutions of sodium thiosulfate (e.g., $Na_2S_2O_3$). As with the chlorine compounds, iodophors are rapidly inactivated by organic matter. One advantage is that iodophors have a built-in indicator. As long as the solution is brown or yellow it is still active.

Phenolic Compounds
These compounds are effective against vegetative bacteria (including *mycobacterium tuberculosis*), fungi, and lipoviruses. Effectiveness against nonlipid viruses is variable depending on the virus. However, phenolic compounds are ineffective against bacterial spores.

Use-dilutions of 1.0 to 5.0 percent, which contain 0.5 to 2.0 percent phenol are effective against lipoviruses.

Phenolic compounds are corrosive and may leave a sticky, gummy residue. They are irritating to the skin and eyes and are relatively toxic (e.g., TLV_{SKIN} is 5 ppm).

Quaternary Ammonium Compounds
The efficiency of quaternary ammonium compounds still generates considerable controversy. They are effective in destroying ordinary vegetative bacteria and lipid- containing viruses but are not effective against pseudomonas, proteus and other gram-negative bacteria. Also, quaternary ammonium compounds are not effective against bacterial spores at the typical use concentrations of 1 to 750 ppm. Use-dilutions of 0.1 to 2.0 percent are recommended.

Quaternary ammonium compounds are surface-active compounds that posses the useful property of lowering the surface tension of the solution. Other advantages include being nontoxic, odorless, nonstaining, noncorrosive to metals and stable. If used at recommended concentrations, they are nonirritating. Quaternary ammonium compounds are rapidly inactivated by organic matter.

Formaldehyde Solutions
Formaldehyde in a 5 to 8 percent concentration is an effective liquid decontaminant that inactivates vegetative bacteria, bacterial spores, lipid and nonlipid viruses and fungi.

The odor, irritation to skin and eyes and toxicity (e.g., TLV of 1.0 ppm) of formaldehyde solutions reduce its desirability for general use. Formaldehyde solutions are active in the presence of organic matter and do not corrode metal.

Use of Disinfectants
Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned the task of making up use-concentrations from stock solutions must be properly informed as to the potential hazards and trained in the safe procedures to follow. The concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Even a small droplet splashed in the eyes may cause blindness. Face shields and goggles should be used for eye protection, and long-sleeved garments and chemically resistant gloves, aprons, and boots should be worn to protect against corrosive and depigmentation effects to the skin. One of the initial sources for hazard information on any given product is the label on the container.

3.10.4 Vapor and Gas Disinfectants

A variety and vapors and gases possess germicidal properties. The most useful of these are formaldehyde and ethylene oxide. When these substances can be used in closed systems and under controlled conditions of temperature and humidity, sterilization can be achieved. Vapor and gas disinfectants are primarily useful in sterilizing:

- Biological safety cabinets (BSCs) and associated air-handling systems and air filters
- Bulky or stationary equipment that resists penetration by liquid disinfectants
- Instruments and optics that might be damaged by other sterilization methods

3.11 Decontamination

Decontamination is the process of physically removing contaminants or changing their chemical nature to innocuous substances. How extensive decontamination must be depends on a number of factors, the most important being the type of contaminants involved. The more harmful the contaminant, the more extensive and thorough decontamination must be. Less harmful contaminants may require less decontamination.

The objective of decontamination is not only the protection of personnel and the environment from exposure to hazardous agents, but also the prevention of contamination of experimental materials. This factor should also be considered in selecting decontamination materials and methods.

3.11.1 General Decontamination Guidelines

Contaminants can be located either on the surface of a material or permeated into the material. Surface contaminants may be easy to detect and remove;

however, contaminants that have permeated a material are difficult or impossible to detect and remove.

Five major factors affect the extent of permeation:

- ***Contact time.*** The longer a contaminant is in contact with an object, the greater the probability and extent of permeation. For this reason, minimizing contact time is one of the most important objectives of a decontamination program. Additionally, if laboratory personnel are splashed, make every effort to remove the contamination as soon as possible.

- ***Concentration.*** Molecules migrate from areas of high concentration to areas of low concentration. As concentrations of hazardous materials increase, the potential for permeation increases.

- ***Temperature.*** An increase in temperature generally increases the permeation rate of the contaminants.

- ***Size of contaminant molecules and pore space.*** Permeation increases as the contaminant molecule becomes smaller, and as the pore space of the material to be permeated increases.

- ***Physical state of hazardous materials and wastes.*** As a rule, gases, vapors, and low viscosity liquids tend to permeate more rapidly than high-viscosity liquids or solids.

Decontamination methods vary in their effectiveness for removing different substances. The effectiveness of any decontamination method should be assessed at the beginning of a program and periodically throughout the lifetime of the program. If contaminated materials are not being removed, the decontamination program must be revised. The following methods may be useful in assessing the effectiveness of decontamination.

Visual Observations

There is no reliable test to immediately determine how effective decontamination is. In some cases, effectiveness can be estimated by visual observation using natural or ultraviolet light.

- ***Natural Light.*** Discolorations, stains, bubbling, corrosive effects, visible dirt, or alterations in clothing fabric may indicate that contaminants have not been removed. However, not all contaminants leave visible traces; many contaminants can permeate clothing and are not easily observed.

- ***Ultraviolet light.*** Certain contaminants, such as polycyclic aromatic hydrocarbons, which are common in many refined oils and solvent wastes, fluoresce and can be visually detected when exposed to ultraviolet light. Ultraviolet light can be used to observe contamination of skin, clothing, and equipment; however, certain areas of the skin may fluoresce naturally, thereby introducing an uncertainty into the test. In addition, use of ultraviolet light can increase the risk of skin cancer and eye damage; therefore, a qualified health professional should assess the benefits and risks associated with ultraviolet light prior to its use.

Wipe Sampling
Wipe sampling provides after-the-fact information on the effectiveness of decontamination. In this procedure, a dry or wet cloth, glass-fiber filter paper, or swab is wiped over the surface of the potentially-contaminated object and is then analyzed in a laboratory. Both inner and outer layers of protective clothing should be tested. Skin may also be tested using wipe samples. Use of pH paper is a common method for checking the “cleanliness” of equipment that has been exposed to corrosives.

Cleaning Solution Analysis
Another method used to test the effectiveness of decontamination procedures is to analyze the cleaning solutions. Elevated levels of contaminants in the final rinse solution may suggest that additional cleaning and rinsing are needed.

Decontamination Solution
Personal protective equipment, surfaces, and laboratory equipment are usually decontaminated by scrubbing with detergent-water followed by rinsing with a sufficient amount of water. While this process may not be fully effective in removing some contaminants (or in a few cases, contaminants may react with water), it is a relatively safe option compared with using a chemical decontaminating solution. A decontamination chemical is intended to change the contaminant into a less harmful substance. Especially troublesome are mixtures from a variety of known or unknown substances. The appropriate decontamination solution must be selected in consultation with qualified experts.

It is important that decontamination personnel understand the potential hazards of the contaminants, as well as any hazards associated with cleaning equipment or special decontamination solutions.

Common decontamination solutions include aqueous solutions, usually 5 to 10 percent, of sodium carbonate, sodium bicarbonate, trisodium phosphate, and calcium hypochlorite.

Additional specialized decontaminants or neutralizing agents that may be considered include: hexane, ethanol, acetone and solvents such as 1,1,1-trichloroethane for small items used in sampling; supertropical bleach (STB); DS2, a mixture of diethylenetriamine (70%), ethylene glycol monmethyl ether (28%), and sodium hydroxide (2%); sodium hydroxide (caustic soda); chelating agents such as ETDA, citric acid, tartaric acid and oxalic acid.

Biological contaminants have been decontaminated using: betapropiolactone (BPL); formaldehyde solution; ethylene oxide-fluorinated hydrocarbon mixture; peracetic acid; and strong bleaches and caustics. These decontaminants all require special care in their handling and use. In addition, many can degrade rubber products which may be found on heavy equipment.

3.11.2 Glassware Decontamination

Laboratories using chromic acid for cleaning laboratory glassware should begin to abandon the practice due to limitations on the concentration of chromium in waste-water discharge and elevated costs for proper treatment and disposal of the spent cleaning product.

Laboratories should seek alternative glassware cleaning solutions to meet their needs. Glassware cleaning solutions that do not contain chromium are readily available. Nochromix™ mixed with sulfuric acid is as effective as Chromerge™ in removing trace metals and enzyme residues, but it eliminates the need for special handling caused by the toxicity of Chromerge™. Purchasers of reagents and chemicals for the affected laboratories should determine if their suppliers can provide such environmentally suitable glassware cleaning agents.

As an alternative to a sulfuric acid bath, a 95 percent ethanol/hydrochloric acid bath or 95 percent ethanol/potassium hydroxide bath can be used effectively against organic residues. In cases where it is imperative that glassware must be cleaned using a solution containing chromium, the spent cleaning reagent should be classified as a hazardous waste.

3.11.3 Biological Decontamination

Figure F2-6 presents some general guidelines for performing biological decontamination.

In addition to the general guidelines, personnel should observe the following procedures when performing decontamination after a spill:

- Consider the use of impermeable aprons/garments and boot covers if the amount of contamination is significant

- Clean up and remove all visible material first, using disposable towels or other means that prevents direct skin contact with the blood or other potentially infectious material (OPIM)

- Place soiled toweling immediately in a leak-proof bag to prevent contamination of other surfaces

- Decontaminate the entire area with clean towels and a 1:10 solution of common household bleach and water

- Collect contaminated items (e.g., paper towels, gloves, etc.) when decontamination is complete and place into a labeled leak-proof bag for disposal

The following sections discuss biological decontamination of equipment; bins, pails, and cans; and biosafety cabinets (inside and outside)

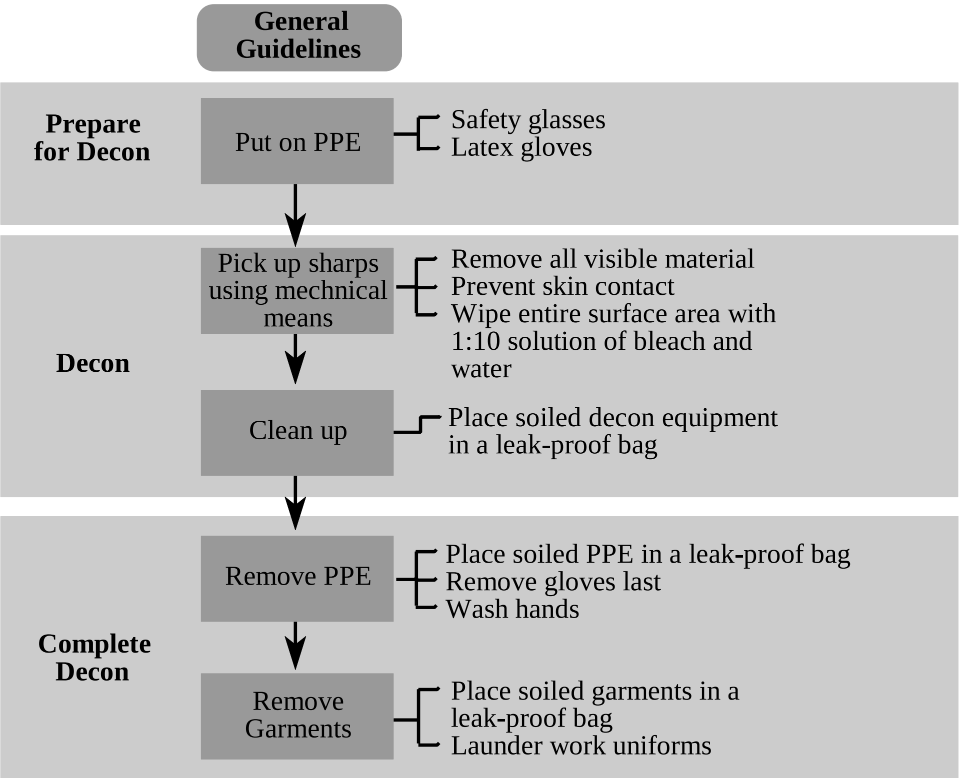
Decontamination of Equipment

Contaminated equipment should be washed thoroughly with a 1:10 solution of common household bleach and water. Contaminated towels and waste must be placed in a leak-proof bag for disposal.

Bins, Pails, Cans

All reusable receptacles (e.g., bins, pails, cans) that have a reasonable likelihood for becoming contaminated with blood

Figure F2-6: General Guidelines for Biological Decontamination



or OPIM, must be inspected and decontaminated on a regular basis. They should be cleaned and decontaminated immediately, or as soon as feasible, upon visible contamination.

All equipment and environmental surfaces must be decontaminated after contact with blood or OPIM at the end of a procedure or work shift, if contamination may have occurred.

Biohazard Spills Inside BSCs

The occurrence of a spill in a BSC poses less of a problem than a spill in an open laboratory as long as the spilled material is contained in the BSC. Decontamination of the work zone can usually be effected by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Gaseous decontamination may be required to clean up the interior sections of the BSC.

Chemical decontamination procedures should be initiated immediately while the BSC continues to operate. Continuing the operation of the BSC helps to prevent the escape of containments from the cabinet.

While wearing protective gloves, spray or wipe walls, work surfaces, and equipment with an appropriate decontamination solution. A disinfectant detergent, such as Wescodyne™ or Environ™ has the advantage of detergent action on extraneous organic substances that may interfere with the microbicidal activity of the disinfectant.

Flood the tray top, drain pans, and catch basins below work surface with decontaminating solution and allow to stand for 20 minutes.

Drain excess decontamination solution from the tray and drain pans into the cabinet base. Lift out the tray and the removable exhaust grille work. Clean the top and bottom (e.g., underside) surfaces using a sponge or clean cloth soaked in decontamination solution. Following the cleaning process, replace the tray and exhaust grille work in their proper position. Place gloves and sponge or cloth in the autoclave pan and autoclave these items.

Drain the decontaminating solution from the cabinet base into an appropriate container and autoclave according to standard procedures.

If gaseous decontamination of the cabinet's interior sections is needed, call the BSO or SHEMP Manager. Figure F2-7 presents a schematic of this process.

Biohazardous Spills Outside BSCs

The protocol to be used in cleaning up of spills involving microorganisms will depend on the amount of material spilled and the degree of laboratory containment required.

If individuals believe that their outer garments have been contaminated, they should remove their clothing in the laboratory area and place them in an autoclave or a container for autoclaving. They should change into a clean clothing in a non-contaminated area. All laboratory personnel should keep a complete change of clothing, including shoes at the laboratory in case of spills.

Special care in decontamination may be necessary if a spill goes under or between fixed furniture or behind base moldings (e.g., floor/wall), or if floor penetrations are involved.

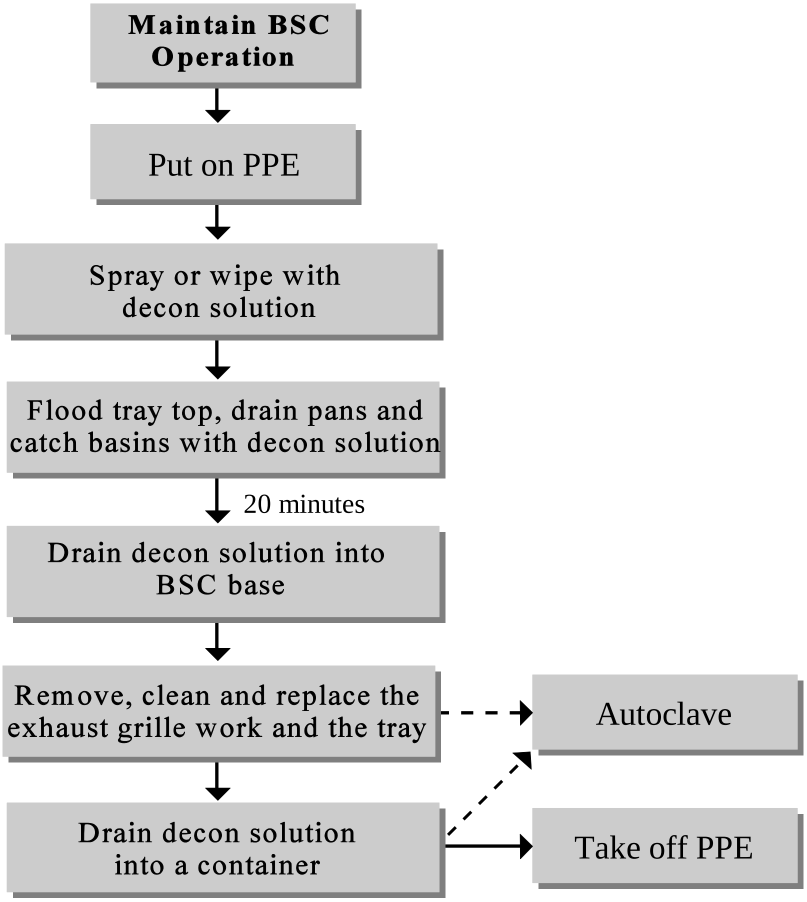
This procedure for minor spills of Class 2 Organisms is shown in Figure F2-8.

Minor Spills of Class 2 Organisms

For minor spills (e.g., less than 10 milliliters and generating little aerosol) of Class 2 Organisms on equipment, laboratory benches, walls, or floors perform the following:

- Warn all personnel not essential for spill containment to stay clear of the contaminated area. This may be accomplished verbally or, when appropriate, by posting warning signs on the doors.
- Thoroughly wash hands and other apparently contaminated areas with soap and water. Put on clean disposable gloves.
- Cover the spill area with paper towels soaked in appropriate decontamination solution.
- Wipe up the spill with the soaked paper towels and place the used towels in an autoclave pan. Autoclave the used towels.

Figure F2-7: Chemical Decontamination of a BSC



- Pour decontamination solution around and on the area of the spill. Let it stand for 20 minutes and then wipe it up with paper towels. Place gloves and paper towels in an autoclave pan and then autoclave.

- Wash hands and other apparently contaminated areas again with soap and water.

Major Spills of Class 2 Organisms
For major spills (e.g., more than 10 milliliters or with considerable aerosol) of Class 2 organisms in a laboratory, perform the following:

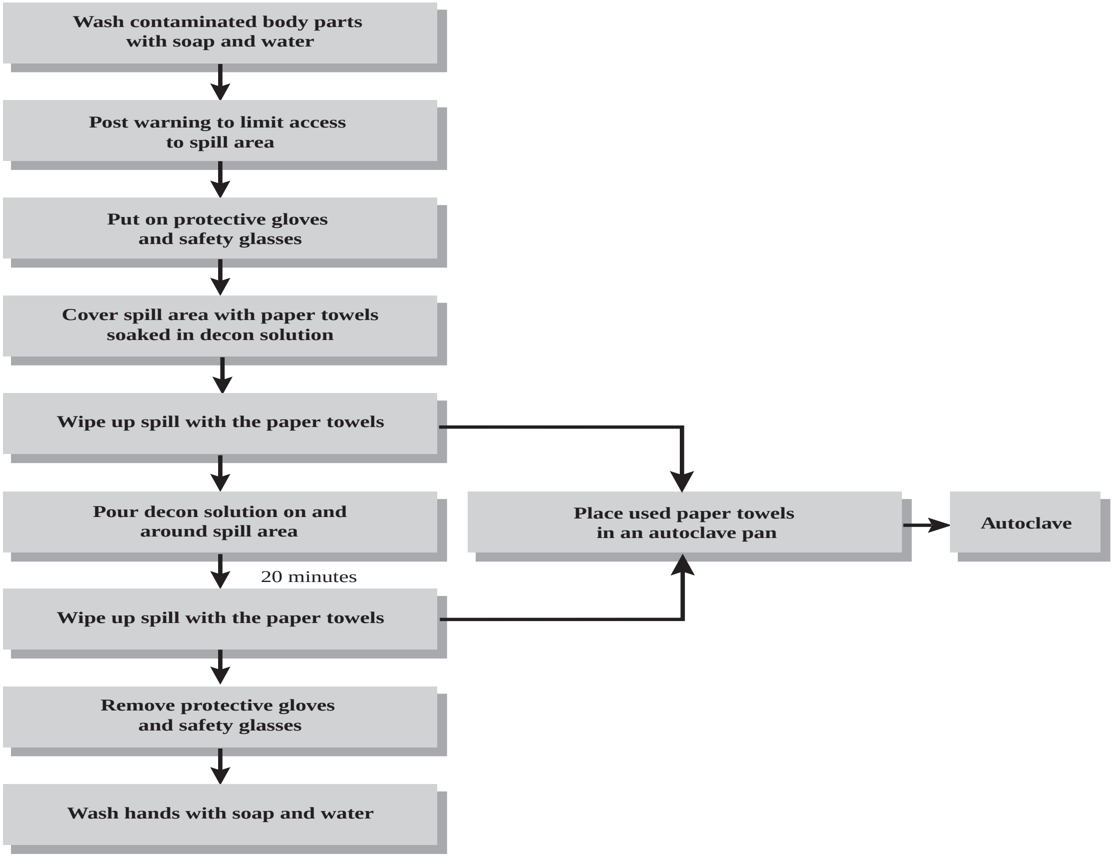
- Close laboratory doors and post warning signs to prevent other employees from entering the laboratory.

- Wash hands and other apparently contaminated areas with soap and water.

- Report the accident to the laboratory supervisor and to the SHEMP Manager.

- If personal clothing is contaminated, remove all outer clothing and place it in an autoclave plan or other container for autoclaving. Put on clean garments.

Figure F2-8: Spill Cleanup Procedure Outside BSCs—Minor Spills of Class 2 Organisms



- Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.

- Upon returning to the laboratory to start decontamination, check to see if laboratory doors are closed and appropriate signs are displayed. Put on surgical gloves. Respirators or other safety equipment may be required, depending on the microorganism involved. Check with the laboratory supervisor or SHEMP Manager.

- Pour a decontamination solution around the spill and allow this solution to flow into the spill. Paper towels soaked with decontamination solution may be used to cover the area. Do not pour decontamination solution directly onto the spill to avoid additional release of aerosols.

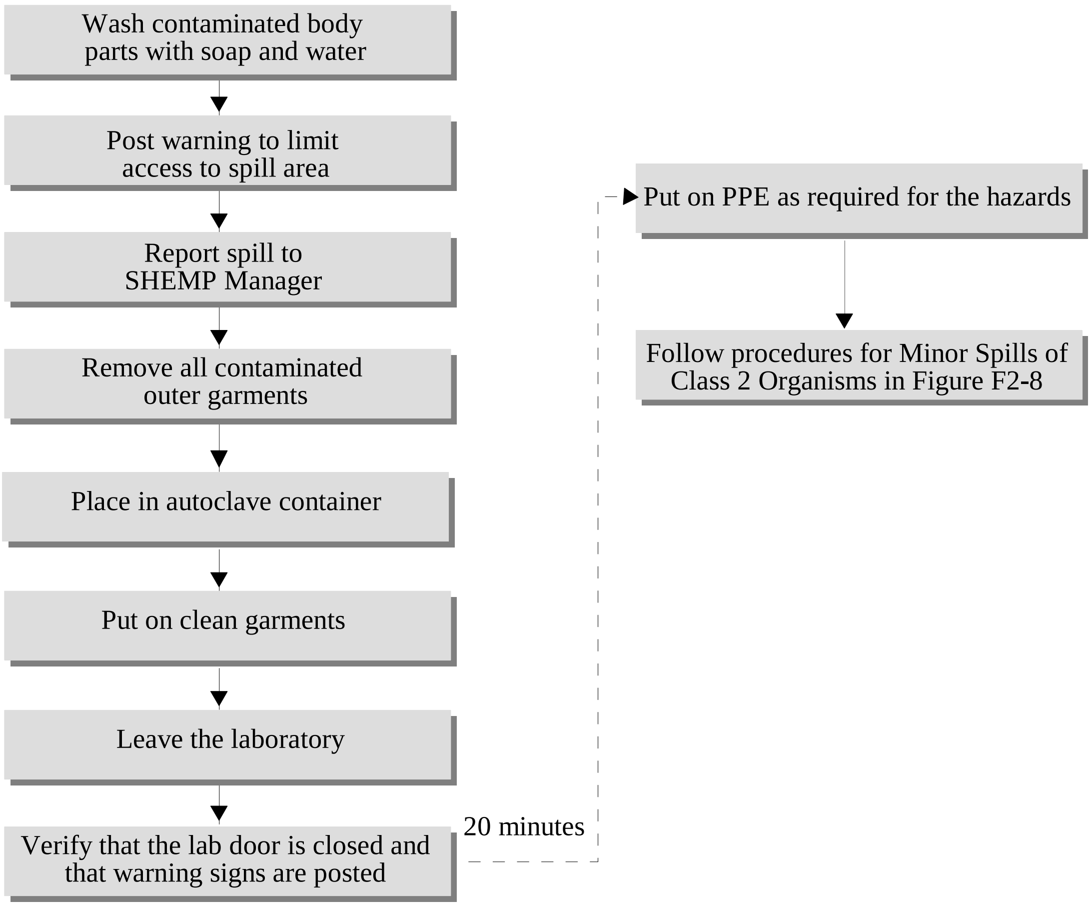
- Let decontamination solution—spill mixture stand for 20 minutes or longer to allow adequate contact time.

- Using a dust pan and squeegee, transfer all contaminated materials to a deep autoclave pan, cover it with a suitable cover, and autoclave according to standard directions.

- Place dust pan and squeegee in an autoclave bag and autoclave according to standard directions.
- Remove gloves and other contaminated garments and place them in an autoclave container for autoclaving.
- Thoroughly wash hands, face, and other apparently contaminated areas.

Special care in decontamination must be taken. The biosafety officer and/or the SHEMP Manager may require the collection of sample cultures to determine that the area has been effectively decontaminated. This procedure for major spills of Class 2 and 3 organisms is shown in Figure F2-9. Refer to Chapter C7 for more information on biosafety**.**

Figure F2-9: Spill Cleanup Procedure for Outside BSCs—Major Spills of Class 2 and 3 Organisms



1.0 Introduction

Among the many risks to worker safety and health that arise through EPA laboratory activities, chemical handling operations, as well as the storage and transportation of chemicals, represent key areas of potential exposure. For any laboratory activity in which chemicals are handled or used, laboratory management must recognize—and address—the risks associated with the physical and health hazards of chemicals, including chemical incompatibilities. The mixing of incompatible chemicals, for example, can result in sudden, violent and unforeseen hazards, and may cause significant personal injury and property damage.

This section outlines the fundamental approach to chemical work practice controls through widely-recognized techniques for minimizing risks associated with receiving, distributing, storing, transporting, and handling hazardous chemicals. For example, microscale experiments are being used by some EPA laboratories as a method to reduce exposure potential to hazardous chemicals.

These work practices, along with the appropriate engineering controls and personal protective equipment, should be implemented by all EPA laboratories to ensure a safe work environment.

EPA Program Requirements

To promote and ensure safe use and handling of laboratory chemicals, each laboratory must ensure that:

- Standard operating procedures for chemical use and handling have been developed and implemented.
- Work areas have been surveyed to determine proper implementation of work practice controls for chemicals.
- Employees have been trained in work practice controls for chemicals.
- Facilities have been inspected to verify appropriate storage for incompatibles.
- Regulatory requirements for transport and disposal of chemicals are met.

Program Administration

The use of chemicals in the laboratory can be effectively managed through the use of work practice controls. Responsibilities should be assigned to:

- Develop standard operating procedures for the procurement and distribution of hazardous chemicals in the laboratory.

- Monitor general work practice controls for chemicals.

- Train laboratory employees on the proper use and handling of hazardous chemicals, including corrosives, flammables, toxics, reactives, and compressed gases.

- Determine whether storage of hazardous chemicals is appropriate and take into account all special considerations for incompatibility, flammability, and peroxide formation.

- Verify that hazardous chemicals are transported and disposed of according to regulatory requirements.

2.0 Procurement and Distribution

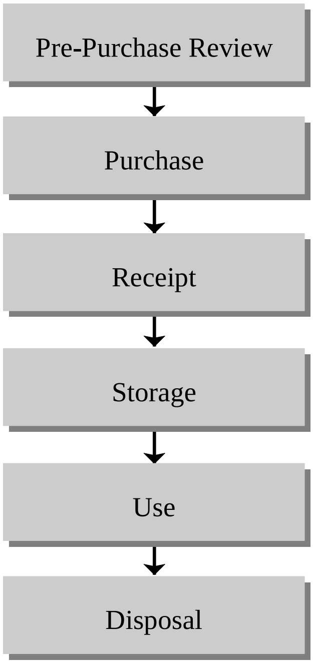
Methods of procurement, receipt, and distribution of hazardous chemicals may vary widely among different laboratories, and may be highly dependent on the size and complexity of the organization, as well as the degree to which its procurement systems are formalized.

However, every laboratory should establish a means by which chemical purchases and deliveries can be reviewed and approved. A pre-purchase review, for example, can be used to evaluate new hazards introduced by procurement of a chemical not previously used at the facility. A pre-purchase review can also be used to minimize the quantities of chemicals purchased, thereby reducing the magnitude of risk. Minimum quantities of chemicals, consistent with normal laboratory needs and requirements, should be maintained.

Before a substance is received, laboratory management should ensure that information on its proper handling, storage, and disposal has been provided to those who will handle the substance. A mechanism should be established, through staff responsible for receiving chemicals, to ensure that no chemical container is accepted without an adequate identifying label or material safety data sheet (MSDS). If the facility or individual users of chemicals maintain a chemical inventory, new chemicals should be entered in the inventory at the time of chemical receipt.

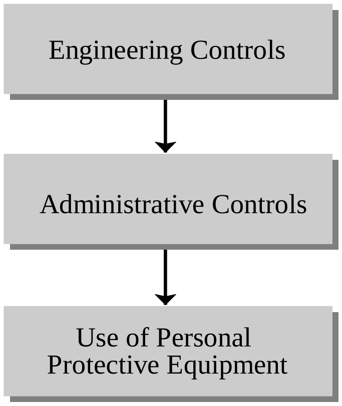
This general procurement process is shown in Figure F3-1.

Figure F3-1: Chemical Procurement and Distribution



3.0 General Work Practice Controls for Chemicals

As with the control of other safety and health hazards, control of chemical hazards should adhere to the hierarchy of control:



Both health and physical risks associated with hazardous chemicals need to be considered when establishing storage and handling guidelines. For example, local exhaust ventilation systems can be used to prevent employee exposures to solvent vapors, and to avoid the creation of flammable or combustible atmospheres in the work area. Physical and health hazards unique to particular chemicals must also be adequately controlled through specific handling and storage methods selected for the work site. In general, the use of the smallest quantity of chemical necessary can help control the magnitude of chemical hazards.

Fundamental chemical hygiene practices should also be observed to prevent chemical ingestion, regardless of the type and quantity of chemicals used in the laboratory.

Refer to Chapter F2 for more information on general work practice controls.

4.0 Chemical Use and Handling

Among the many tasks and operations performed daily by laboratory employees, those involving direct handling and/or transport of hazardous chemicals pose the greatest potential for exposure. Procedures that may produce aerosols, including particulates and mists as well as vapors and gases, must be conducted in ways that minimize the generation of air contaminants. Sound chemical-handling practices also help minimize other forms of potential exposure (e.g., through the skin). The following common laboratory chemical operations are typically associated with higher exposure risk:

- Weighing, transferring, pouring, siphoning
- Mixing, blending, shaking
- Stirring and vortexing

Even when mechanical methods are employed to conduct these operations, laboratory management and employees must always anticipate the possibility of mechanical failure, and be prepared for an unexpected release of hazardous materials.

Specialized handling precautions and good laboratory practices have been developed for specific classes of chemical and physical hazards. The following guidelines should be used in conjunction with information on chemical and physical hazards associated with hazardous chemicals used at laboratory facilities. Chemicals of any hazard class should be used only if the quality of the available ventilation system(s) is appropriate. Personal protective equipment should be worn during all operations that require chemical handling.

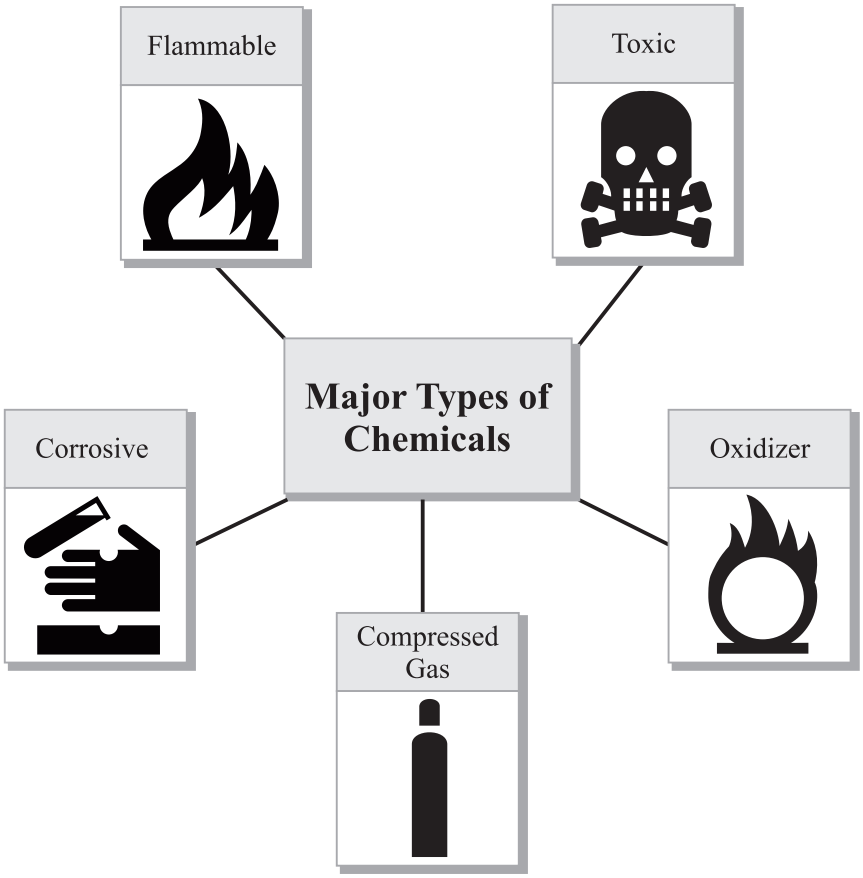
Figure F3-2 presents a summary of the major types of chemicals discussed in this chapter.

4.1 Corrosives

The following controls and handling techniques should be employed when handling corrosives (acids and bases):

- As applicable, wear appropriate personal protective clothing (e.g., acid-resistant apron, chemical-resistant gloves, splash goggles and face shield).

Figure F3-2: Major Types of Chemicals Used and Handled at EPA Laboratories



- Conduct the procedure in a laboratory fume hood.
- Use proper pouring techniques when pouring acids into water.
- Perform all dilutions of corrosives in a laboratory fume hood.

4.2 Flammables

The following controls and handling techniques should be employed when handling flammables:

- Keep flammable compound(s) away from ignition sources, such as open flame.
- Conduct procedure in a laboratory fume hood, especially while transferring chemicals from one container to another or heating chemicals in an open container.
- Heat flammable substances in steam, water, oil, hot air baths, or heating mantle.
- When flammable liquids are transferred in metal equipment, minimize generation of static sparks by using bonding and grounding straps as appropriate.

4.3 Toxics

If a less-toxic substance cannot be substituted, there are general safeguards available to provide protection:

- Check with other labs performing similar research to investigate the procedures and safety precautions they are using.
- Have the needed toxic material transferred from other labs instead of purchasing additional material.
- Always work with the smallest amount of material possible.
- Label all containers with the chemical name and warning of the material's potential hazards.

SHEM Guide 24, "Chemical Hygiene," contains additional information on working with particularly hazardous substances.

In addition to these general safeguards, below are specific recommendations for personal protective equipment (PPE), hoods, spills, and first aid.

4.3.1 Use of PPE

Appropriate PPE must be used, including impervious gloves, safety glasses with solid side shields, and a fully-fastened laboratory coat or other protective clothing. Double gloving is highly recommended when working with toxic materials. This involves wearing an inner glove of a particular material that is resistant to the chemicals in use, and wearing an outer pair of gloves of a different material that is also impervious to the chemicals. This is a good practice to use at all times, but is particularly important when working with highly toxic materials that are easily absorbed through the skin.

4.3.2 Use of Fume Hoods

Work with toxic materials must be performed in a chemical fume hood. Work with the material should be performed at least six inches into the hood. Before beginning work, personnel must verify that the hood has a current certification sticker and is in good working condition. If any problems with the operation of the hood is noted, the SHEMP Manager must be contacted immediately.

4.3.3 Toxic Material Spill Cleanup

In the event of a spill, the materials and the area must be cleaned promptly. The materials' MSDS should be checked to verify use of proper types of cleaning materials and procedures. If any questions arise, the SHEMP Manager should be contacted. The used cleanup material must be safely and promptly disposed of.

4.3.4 First Aid for Toxic Material Exposure

Laboratory personnel should know not only the toxicity of the material they are working with, but also the first-aid procedures needed in case of exposure. If an antidote or special first-aid procedure is required, it must be verified that the medical provider has the provisions available to accommodate potential emergency needs. Knowledge of the hazardous properties of materials and proper response actions must be communicated to anyone working in areas where toxics are used.

4.3.5 Specific Examples of Toxic Materials

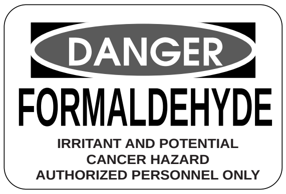
The following sections present specific work practice controls for use of such toxic materials as dioxin, formaldehyde, methylene chloride, polychlorinated biphenyls (PCBs), and pesticides. Figure F3-3 summarizes these toxic materials and shows typical signs related to their use.

Dioxins

Dioxins must be handled as "particularly hazardous substances," as outlined in SHEM Guide 24, "Chemical Hygiene."

Formaldehyde

For work with formaldehyde, in addition to the general safeguards outlined above, specific work practices must be followed. A regulated area must be established for any locations with formaldehyde concentrations exceeding the time-weighted average (TWA) or short-term exposure limit (STEL). The regulated area must bear a sign with the following information:

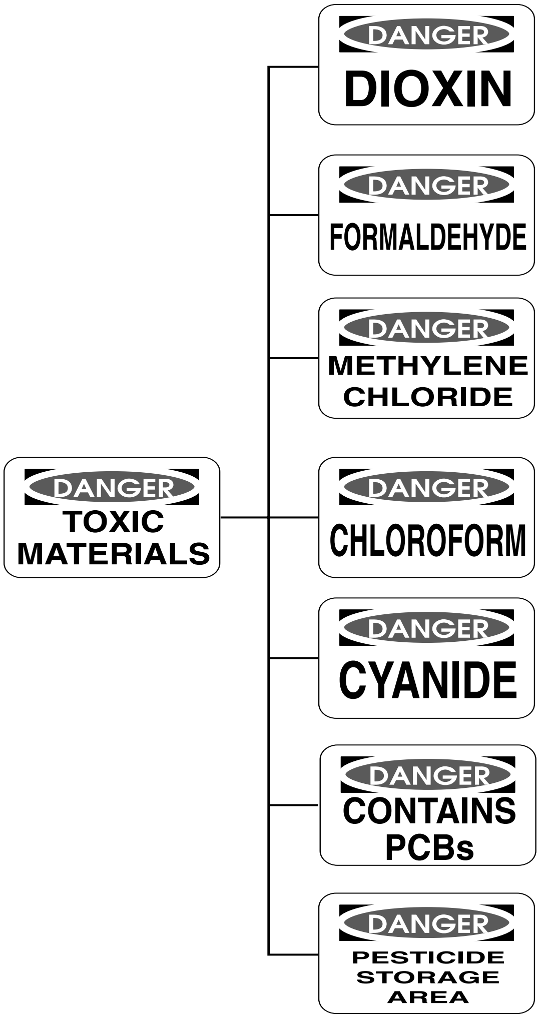


Work with formaldehyde must be performed in a fume hood. Employees must be trained specifically for work with formaldehyde. The facility's chemical hygiene plan (CHP) may contain additional information on laboratory work with formaldehyde.

Methylene Chloride

A regulated area must also be established for locations with methylene chloride concentrations exceeding the TWA or STEL. The area must be marked with the appropriate hazard warning. Work must be performed in a fume hood. Employees must be trained specifically for work with methylene chloride. The facility's CHP may contain additional information on laboratory work with methylene chloride.

Figure F3-3: Typical Signage for Toxic Materials



Chloroform

During the use of chloroform, a hazard can be presented by preserving chloroform with alkenes. Spontaneous generation of phosgene gas may occur. Laboratory personnel should not store chloroform in this manner. In addition, all old alkene-preserved chloroform bottles should be tested for phosgene. When wetted with five percent diphenylamine and five percent dimethyl-aminobenzaldehyde, then dried, filter paper strips turn yellow in the presence of phosgene vapor.

Cyanide

Employees working with cyanide must follow the safe work practices outlined in the EPA Standard Operating Procedure (SOP) for cyanide. Researchers performing cyanide analyses are required to read the SOP and MSDSs of all materials involved before beginning an analysis. High levels of cyanide may be present in some samples analyzed at EPA laboratories, and it is possible for hydrogen cyanide and cyanogen chloride to be released during analyses. Extreme care must be taken when handling cyanide-containing substances, as well as the reagents used in sample preparation.

The work practices outlined below must be followed when working with cyanide:

- *Medical surveillance.* Any employee working with cyanide must be included in a medical surveillance program, including a baseline medical evaluation and an annual exam thereafter.

- *Training.* All staff members working with cyanide must be trained in cardiopulmonary resuscitation (CPR). Training must be documented and updated each year.

- *Labeling.* Containers of cyanide solutions or waste must be labeled as containing cyanide. Solutions and standards must be labeled with the following:
  - — Cyanide
  - — Additional contents (list must be specific)
  - — Hazard warnings
  - — Date of preparation
  - — Date of expiration
  - — Researcher’s initials

- *Waste Disposal.* Waste disposal must be documented into an electronic waste disposal documentation system and a log book. Cyanide waste containers must have the following information on the label:
  - — Cyanide
  - — Additional contents (list must be specific)
  - — Physical state of the waste
  - — Accumulation start date
  - — The words “Hazardous Waste”
  - — Hazard warnings
  - — Name and address of the generator

- *PPE.* A fully-fastened laboratory coat, gloves, and safety glasses must be worn at all times when working with cyanide solutions. All cyanide distillation procedures must be conducted in the fume hood with the sash completely closed. Personnel who enter the laboratory while cyanide analysis is being performed should be cautioned that the analysis is taking place and informed of the potential hazards.

- *Housekeeping.* After an activity is completed, all work areas must be cleaned by disposing of any used and unwanted materials and supplies. Equipment, materials, and supplies must be cleaned immediately after use and returned to proper storage. Hands must be washed prior to leaving the laboratory, after analyses are complete.

- *Accidental Spills.* In the case of an accident involving cyanide, personnel must notify the SHEMP Manager at the laboratory as soon as possible. For a major cyanide spill in the laboratory, all personnel must immediately evacuate the room, locking the doors on the way out. The SHEMP Manager and the fire department must be notified immediately.

PCBs

Items containing PCBs must be marked with the label:

For example, the following items must be labeled:

- PCB containers
- PCB article containers
- Each storage area used to store PCBs and PCB items for disposal
- PCB equipment containing PCBs
- PCB items containing PCBs in concentrations of 50 to 500 ppm
- Any chemical substance or mixture that is manufactured and that contains less than 500 ppm PCB

Laboratory personnel should always order the lowest concentration required for PCB research projects, since waste solutions are characterized by their initial concentration of the PCB, and PCB materials with a concentration of less than 50 ppm may be characterized as *excluded PCB product* for waste disposal purposes. PCB waste must be transported off-site to an approved waste management facility.

Pesticides

Pesticides should be handled as “particularly hazardous substances,” as outlined in SHEM Guide 24, “Chemical Hygiene.” Handling procedures are as follows:

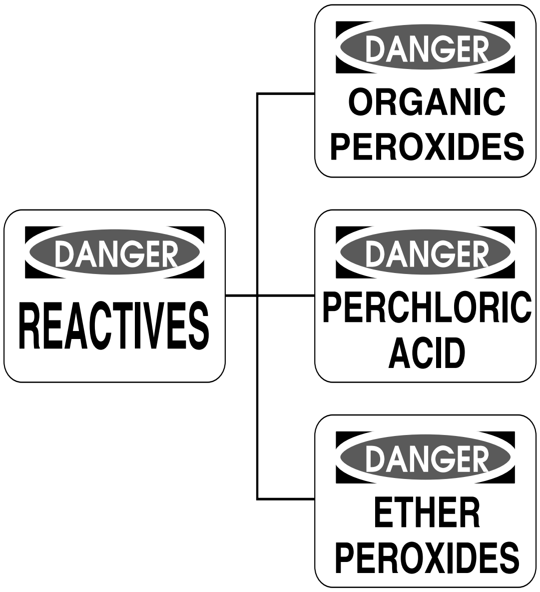
- Establish a designated area for pesticide handling.
- Use containment and control devices (e.g., fume hoods, secondary containment).
- Use the smallest amount of pesticide-containing material as possible.
- Provide training specialized for pesticide handling.
- Use appropriate protective equipment (absorption through skin is a primary pesticide hazard).
- Wash hands and exposed areas immediately after handling.
- Follow established equipment decontamination procedures.
- Keep records of amounts of pesticides stored and used; include dates and names of users.
- Follow established procedures for safe removal of contaminated waste.

4.4 Reactives

A routine chemical reaction is one that occurs at a slow reaction rate and can be easily controlled. However, reactive chemicals can lead to reaction rates that differ greatly. Such reactions can be initiated by elevated temperatures, others by mechanical shock or pressure. Many reactive chemicals have little warning of shock sensitivity, if any, on bottle labels. This section presents work practice controls for commonly used reactives, including organic peroxides, perchloric acid, and ether peroxides. However, there are others, such as nitrates and persulfates, that can be shock-sensitive. In addition, azide solutions should NOT be poured down the drain because they form shock-sensitive salts on metallic plumbing systems.

Figure F3-4 presents a summary of the reactive materials discussed here with typical signage associated with their use.

Figure F3-4: Typical Signage for Reactive Materials



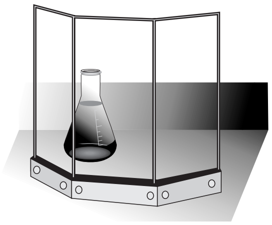
4.4.1 Organic Peroxides

Organic peroxides have unusual stability problems that make them among the most hazardous of substances handled in the laboratory. As a chemical class, these materials are low-power explosives and are hazardous because of their extreme sensitivity to shock, sparks, heat, friction, light, and strong oxidizing and reducing agents.

Peroxides have a specific half-life, or rate of decomposition, under any given set of conditions. Before using a peroxide or potential peroxide-forming material (i.e., ethyl ether, picric acid), personnel should find out all they can about the material, its hazards, and the conditions causing the decomposition.

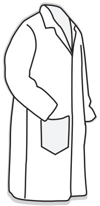
Work must be planned carefully so that only small quantities of materials will be used. A micro-reaction can yield adequate information and product. In bulk quantities, a material's low rate of decomposition may autoaccelerate and cause a violent explosion.

Metal spatulas or metal containment pans should not be used with organic peroxides, because contamination by metals can lead to explosive decomposition. Ceramic implements and containment pans should be used. Work should be performed around a good table shield so that it is between the employee and the operation. A 0.25-inch-thick acrylic shield is recommended for use with a maximum 5-gram quantity detonation. However, this is not effective against metal shrapnel.

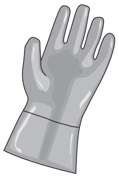


If more than a few grams of unstable material must be handled, or if containment in metal equipment is contemplated, a barricaded cell should be provided with operations conducted by remote control.

Appropriate PPE must always be used for work with organic peroxides:

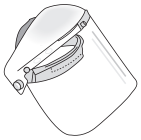


Long-sleeve, fully-fastened laboratory coat



Protective gloves

Safety glasses with solid sideshields



Face shield (preferably with a snap-on throat protector)

Apparatus that is easily controlled should be used so that rapid elevations of temperature or pressure are avoided. For example, an oil bath heater with redundant controls helps guarantee that the desired temperature will not be exceeded.

Personnel must be alert to signs that an operation is not going as expected and be prepared to reduce the heat, quench the reactants, or dump the contents—usually into water.

Organic peroxides should be discarded if their expiration dates have been reached, even if they have been stored for less than a year.

4.4.2 Perchloric Acid

Some analytical procedures involve treating residues with perchloric acid. This acid is a powerful oxidizing agent that may react explosively with reducing agents and organic compounds. Because this reagent combines with oil and other combustibles to form explosive perchlorates, the work must be done in a special perchloric acid fume hood equipped with wash-down facilities to remove the water-soluble materials from all contacted surfaces. If work with these materials is anticipated, the SHEMP Manager must be contacted so that proper safety precautions can be taken.

Strong oxidizing agents should be stored and used in glass containers. Corks or rubber stoppers should not be used. Primary containers should be kept on glass or ceramic trays that have enough volume to hold all the material in the container should it break.

4.4.3 Ether Peroxides

Diethyl ether, isopropyl ether, dioxane, and many other common laboratory materials can form explosive peroxides when stored or evaporated in the presence of air. Although ethers can be tested for the presence of peroxides, which can be removed by proper treatment, preventing peroxide formation is the preferred method of control. Peroxide-forming materials are listed in three categories in Table F3-1.

Table F3-1: Peroxide Formation Potential

Category A	Category B	Category C
Peroxides may form in storage	Peroxides may form during concentration	Peroxides may form during polymerization
Isopropyl ether Divinyl acetylene Vinylidene chloride Potassium metal Sodium amide	Diethyl ether Tetrahydrofuran Dioxane Glyme Vinyl ethers Dicyclopentadiene Diacetylene Methyl acetylene Decahydronaphthalene Tetrahydronapthalene Cyclohexane Diglyme	Methyl methacrylate Styrene Acrylic acid Acrylonitrile Vinylidene chloride Tetrafluoroethylene Vinyl acetylene Chlorotrifluoroethylene Vinyl acetate Vinyl chloride Vinyl pyridine Chloroprene

The lists are broken down by the manner in which the peroxides are formed. When any material listed in category A or B of this table is purchased, each container must be labeled with the date of receipt. If a peroxide analysis is obtained, the result should also be entered on each container. Unopened cans or bottles should be discarded one year after receipt.

Upon opening a can or bottle, the date opened must be entered on the label. All containers must be discarded six months after opening. All containers of doubtful history or condition should be discarded. Crystals seen in ether may be peroxides. Laboratory personnel must not agitate the container or loosen the cap. The SHEMP Manager should be contacted for assistance with disposal.

Ether solutions should not be evaporated or distilled to dryness. Ten percent of the volume should be left in the container. An air stream should never be used to evaporate or agitate ether—nitrogen must be used carefully. A table shield should be used for protection when evaporating or distilling ether or other materials noted in Table F3-1. A long-sleeved laboratory coat, protective gloves, safety glasses with solid sideshields, and a face shield (preferably with a snap-on throat protector) must be worn at all times when handling these materials.

4.5 Compressed Gases

Compressed gases may present a unique, simultaneous mechanical and chemical hazard. Release of flammable gases presents the danger of fire or explosion. Hazards can arise from the reactivity and toxicity of certain gases. Also, asphyxiation may occur by high concentrations of released gases; even those considered “harmless,” such as nitrogen. The amount of potential energy resulting from vessel pressure can cause the additional hazard presented by a cylinder “torpedo.”

This section provides a summary of some work practice controls for use of all compressed gas cylinders as well as information on specific types.

4.5.1 Storage, Handling and Inspection

Work practices for storage, handling, and inspection of compressed gases are presented in the following sections.

Storage

Compressed gas cylinders must be stored away from excessive heat and at least 20 feet away from highly combustible materials. They must be stored in a secured area where they cannot be damaged or knocked over by passing or falling objects. All cylinders in use and being stored must be secured to a fixed structure. Suitable restraining devices must be supplied in all storage locations.

If stored inside, the cylinders must be in a well-protected, ventilated, and dry location. Only minimum practical quantities of compressed gases should be kept and used inside buildings. It is preferable to store cylinders in suitable outdoor storage areas. Storage locations should be assigned by class of material, as well as whether the cylinder is full or empty. Unless the cylinder is in use, the valve cap must be on both full and empty cylinders at all times.

Handling

Compressed gas cylinders must be moved using a hand cart, with a chain to secure the cylinder. During transport, the steel safety cap must be attached to the top of the cylinder to prevent the valve from being snapped off if the cylinder is dropped. When changing gas cylinders, the safety cap must remain in place until the new cylinder is secured. A safety cap must be snugly screwed onto old cylinders before moving them. An old regulator must never be used for a new application. Gases perfuse the metal in the regulators. A new gas used in an old regulator may react with the old gas as it passes through the regulator. This may cause an explosion. Regulators should always be used for the same application, or changed upon new application.

Inspection

Visual inspections must be performed to determine that compressed gas cylinders are in a safe condition. The threading and airways on both the cylinder and regulator should be checked for damage and cleanliness. The tubing should be checked for leaks and corrosion. The hydro-test date of the cylinder (stamped on the neck) should be checked to ensure the date is within the last five years. Inspections must be conducted as per U.S. Department of Transportation (DOT) regulations, 49 CFR parts 171-179 and 14 CFR part 103. In addition, Compressed Gas Association Pamphlets C-6-1968 and C-8-1962 must be consulted for inspection procedures.

Leak detection is an important component of cylinder inspection. Most leaks will occur at the top of the cylinder in areas such as valve threads, regulator, valve stem, and valve outlet. A flammable-gas leak detector or soapy water (or other suitable solution) should be used for leak detection. Laboratory staff must never attempt to repair a leak at the valve threads or regulator. The supplier must be consulted for instruction. If a leak is detected and cannot be remedied by tightening the valve or a packing nut, emergency action procedures should be followed.

A leak of minimum size can often be handled without serious personnel exposure. If it is necessary to move a leaking cylinder through populated portions of the building, a plastic bag or rubber-type shroud should be placed over the top and taped to confine the leaking gas. The procedures shown in Table F3-2 must then be followed based on gas type.

If the leak constitutes a more serious hazard, self-contained breathing apparatus or protective apparel (or both) may be required. Basic action for large or uncontrolled leaks may include any of the following steps:

- Evacuation
- Rescue of injured personnel by crews equipped with adequate personal protective apparel and breathing apparatus
- Fire-fighting action
- Emergency repair
- Decontamination

Table F3-2: Procedures for Leaking Cylinders Based on Gas Type

Type	Procedure
Flammable, Inert, or Oxidizing Gases 	Move the cylinder to an isolated area away from combustible material.
	Post signs that describe the hazards and state warnings.
Corrosive Gases 	Move the cylinder to an isolated, well-ventilated area.
	Use suitable means to direct the gas into an appropriate chemical neutralizer.
	Post signs that describe the hazards and state warnings.
	Note: The size of the leak may increase as the gases are released.
Toxic Gases 	Move the cylinder to an isolated, well-ventilated area.
	Use suitable means to direct the gas into an appropriate chemical neutralizer.
	Post signs that describe the hazards and state warnings.

The facility must be prepared for any of these actions. Guidelines for controlling and handling compressed gases are summarized in Table F3-3.

4.5.2 Examples of Compressed Gases and Gas Generators

Safety considerations for some common types of gases and gas generators (e.g., cryogenics, ozone and hydrogen generators) are discussed in the following sections.

Cryogenics

Cryogenic fluids are liquefied gases that boil at 212°F (100°C) or below. They are shipped and stored at low pressures in special insulated vessels. Liquid nitrogen and liquid oxygen are commonly used in the laboratory as sources of gas and also as low-temperature cooling media. Liquid hydrogen and liquid helium are also available for specialized laboratory operations. Table F3-4 lists the most commonly used cryogenic materials and their respective boiling points.

Table F3-3: Guidelines for Controlling and Handling Compressed Gases

Use	Regulatory requirements of 29 CFR 1910, Subparts H-M must be met.
Construction	Cylinders must be constructed, charged, shipped and maintained in accordance with DOT specifications and regulations in 49 CFR and OSHA specifications in 29 CFR 1910.101 through 169, Subparts H-M.
Size and number	Depends on system size, room size, construction, ventilation, cylinder contents, and availability of fire suppression mechanisms.
Maximum cubic feet	A single flammable gas or oxygen cylinder must be $\leq$ 220 cubic feet.
	Table 8-2 of NFPA Standard No. 45 defines the number of cylinders allowed in the laboratory (typically 6 cylinders in a sprinklered space and 3 cylinders in a non-sprinklered space).
Inspection	EPA laboratories must verify the safe condition of compressed gas cylinders.
	Visual inspections must be performed pursuant to DOT Hazardous Materials Regulations (49 CFR 171-179).
	Where DOT requirements do not apply, guidance in CGA pamphlets C-1-1968 and C-8-1962 should be followed.
Handling/storage/use	Guidance provided in CGA Pamphlet P-1-1965 must be used.
Ventilation rates	Must be sufficient to prevent gas concentration from reaching the lower explosive limit (LEL) resulting from the leakage of one cylinder.
Pressure relief devices	Must be installed and maintained in accordance with CGA Pamphlets S-1.1-1963 with 1965 Addenda, and S-1.2-1963.
Supply lines	Must be securely fastened or anchored every five feet for high-pressure cylinders.

Table F3-5: Boiling Points for Commonly Used Cryogens

Cryogen	Boiling Point
Oxygen	-290°F (-183°C)
Nitrogen	-32°F (-196°C)
Hydrogen	-423°F (-253°C)
Helium	-452°F (-269°C)

As with gas cylinders, containers of cryogenic materials must be clearly marked as to their contents. For example:

The primary hazards of cryogenic materials are:

- Fire or explosion
- Pressure buildup
- Embrittlement of structural materials
- Destruction of living tissue
- Asphyxiation
- Fire

Each are described in the following sections.

- *Fire or Explosion*. Fire or explosion is of concern when gases such as oxygen are used. Enriched oxygen will greatly increase the flammability of ordinary combustible materials. Noncombustible materials may even be caused to burn readily in the presence of oxygen. Oxygen-saturated wood has been known to literally explode when subjected to shock.
- *Pressure Buildup*. When cryogenic materials are maintained in pressure vessels, adequate ventilation and pressure relief must be provided. First, there is the experimental volume, which could be any space with the experimental device immersed in the cryogen within which cryogenic fluid could leak and later cause excess pressure when the system is warmed up.

  Next is the bath space, or the space above and including the cryogenic fluid. This too must be vented. Last is the vacuum space, which must be independently provided with pressure relief. If a cryogenic fluid is leaking into a vacuum space, no problems will arise as long as the cold fluid is present. However, when the cryogenic fluid is withdrawn, the air or gas that has leaked in can expand, and blow up the vacuum space by imploding in the inner vessel.
- *Embrittlement of Structural Materials.* Embrittlement is a situation where the gas/liquid being stored actually penetrates the material of the cylinder or other container, which can cause a catastrophic failure of the vessel.
- *Destruction of Living Tissue.* Even very brief skin contact with a cryogenic liquid can cause tissue destruction similar to that of thermal burns. Prolonged skin contact can result in blood clots that have potentially serious consequences. For this reason, gloves that are impermeable to the fluid being handled and loose enough to be tossed off easily must be worn when working with cryogenic materials.

If cryogenic materials are dispensed from one cylinder into any other container, gloves and a face shield must be used and remain at the site of dispensing. If the cryogen is piped directly into an instrument or apparatus, the gloves and face shield must be in the immediate vicinity (within the same room).

- *Asphyxiation.* Cryogenic fluids released or spilled in a confined area can rapidly alter the air composition by displacement or contamination. Thus, an asphyxiating or toxic risk is introduced.

  Massive amounts of nitrogen, helium, carbon dioxide, or other inert gases can displace oxygen and asphyxiate people or animals.

- *Fire.* A spill of any cryogenic material boils rapidly and releases very large volumes of the material into the atmosphere, oxygen can cause combustible materials to burn violently or explode.

CRYOGEN SAFETY

DO:
- Wear proper eye and hand protection.
- Work with adequate room ventilation.
- Handle cryogenic fluid containers with great care.
- Keep vents open.
- Watch for a blue color indicating condensed oxygen.

DON'T:
- Shake cryogenic liquid containers, as it stimulates boiling.
- Bump containers.
- Touch cold fittings or lines without gloves.
- Vaporize large quantities of cryogenic liquid in a closed room.

Before working with cryogens, read material on safe handling and discuss the hazards and proper procedures with your supervisor. Refer to Chapter F2 for general work practice controls when using Dewars to hold and transport cryogens.

Ozone Generators

Ozone generators have two principal hazards:
- The toxicity of ozone
- The high voltages required in the process

Ozone should be released only in a hood and should never be sniffed or breathed. The detectable odor level is substantially higher than the concentration acceptable for breathing. Therefore, if it can be smelled, the concentration breathed is too high. The gas should be connected to the apparatus consuming it before the current is turned on.

All cabinet parts and shields must be kept in place when the generator is energized. Before any interior adjustments are made, disconnect the power source and ground of all parts (especially condensers and vacuum tubes), before touching any terminals or other metal parts.

Some ozone compounds are unstable; elevated concentrations should be avoided and quantities in the experiment should be kept as small as possible. A table shield should be used for protection. Ozonides formed at low temperatures may be unstable at room temperature. Therefore, such materials must be kept behind a shield or other protection.

Ozone may react violently with organic materials such as rubber and plastic. All materials used in an apparatus must be acceptable for ozone use and thoroughly degreased before introducing ozone flow.

Hydrogen Generators

Electrolytic cells used to generate hydrogen for chromatography are substantially safer than having a hydrogen cylinder in the room. However, one hazard involves the presence of hydrogen possibly mixed with air or oxygen, particularly at startup or during other unstable conditions.

Ignition sources must be kept away from hydrogen generators or vent locations. The manufacturer's directions should be read carefully before installing or servicing a hydrogen generator. Only the electrolyte specified or furnished by the manufacturer should be used and replaced according to the instructions.

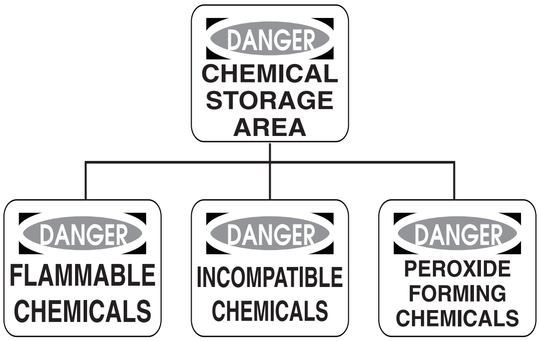
5.0 Chemical Storage

Chemicals in storage should be protected to preclude leaks, spills, and other forms of physical damage (e.g., earthquakes and fire). For this reason, storage on benchtops and in hoods should be avoided. Spill trays, spill- and shatter-proof containers, secondary containers, and proper receptacles should be used as needed. To ensure that chemicals do not deteriorate while stored, they should be properly identified and labeled with date of receipt, opening, and expiration; expired chemicals should be disposed of as soon as possible. Chemicals should also be stored away from direct sunlight and high-heat sources. Water-reactive chemicals should not be stored in storage areas that have sprinklers.

For more information on hazard-specific controls, refer to Chapter D5.

This section provides information on the storage of incompatible flammable and peroxide-forming chemicals, as shown in Figure F3-6.

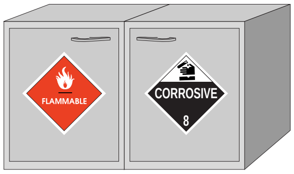
Figure F3-6: Storage of Incompatible, Flammable, and Peroxide-Forming Chemicals



5.1 Incompatible Chemicals

When incompatible chemicals are mixed, whether inadvertently during a chemical manipulation or accidentally in storage, they can react to form compounds or other chemicals, with an attendant consumption or generation of energy. The end-products or byproducts themselves may be hazardous, or the magnitude of energy generated by the mixture may be destructive. A fire, for example, will produce not only light and heat, but also toxic combustion products. Whenever generation of light, heat, or pressure occurs in excessive magnitude, or with excessive speed, an explosion or fire can result, and the effects can be catastrophic. Even reactions that generate an innocuous gas or vapor warrant concern, since significant amounts can displace the available oxygen in an enclosed area and create an oxygen-deficient environment.

To minimize the risks associated with chemical incompatibility, the laboratory should establish a segregation scheme for chemicals in storage to ensure that accidental breakage, leaks, or other destruction of chemical containers do not result if they should react with incompatible materials. Sources such as the chemical incompatibility table (refer to Attachment F3-1), MSDSs, and other references should be consulted for guidance. In addition, some chemical manufacturers color-code their labels according to compatibility to help chemical users readily segregate substances appropriately.



At a minimum, laboratories should segregate acids, bases, oxidizers, and flammable chemicals from one another. Also, chemicals should not be stored alphabetically unless they belong to one segregation class.

5.2 Flammable Chemicals

Numerous guidelines, including the National Fire Protection Association's (NFPA) Standard 45 for laboratories, provide guidance on the storage and handling of flammable liquids. In general, prudent storage practices include the following:

- Flammable liquids (flashpoint less than 100° F) must be stored away from heat and ignition sources.
- Flammable liquids in large quantities should be stored in metal safety cans. The cans should be used only as recommended by the manufacturer.
- Users should:
  - — Never disable the spring-loaded closure.
  - — Always keep the flame arrestor screen in place.
  - — Replace the flame arrestor if it is punctured or damaged.
- If a reagent must be stored in glass for purity, the glass container may be placed in a bottle carrier to lessen the danger of breakage.
- Small quantities (working amounts) of flammable chemicals may be stored on open shelves.

- Flammable chemicals must be stored in flammable-liquid storage cabinets that have been approved by Factory Mutual and/or listed by Underwriters Laboratory and designed in accordance with NFPA Code No. 30. The following safety practices should be observed:

  — Store only compatible materials inside a cabinet.

- — Do not store paper, cardboard, or other combustible packaging material in a flammable-liquid storage cabinet.
- — Do not overload cabinets; follow manufacturers' established quantity limits.
- — Follow NFPA guidelines for maximum allowable volumes.

- Do not store flammables in areas exposed to direct sunlight.
- The quantities of flammable chemicals stored in laboratories should be kept to a minimum.

5.3 Peroxide-Forming Chemicals

If stored or handled improperly, chemicals that can form peroxides may be explosive. The following guidelines should be observed if these chemicals are stored in the laboratory or elsewhere:

- Label peroxide-forming chemicals with the date they were opened.
- Store peroxide-forming chemicals away from heat sources and light.
- Do not use metal containers since some metal oxides can promote the formation of peroxides.
- Use proper antioxidant inhibitors. *[Note: The inhibitor may be consumed with time, making the compound again sensitive to peroxidation.]*
- Test peroxide-forming chemicals for peroxides every three months using test paper strips (dietheyl and diisopropyl ether should be tested on a monthly basis). If the test is positive, the material must be treated to remove the peroxides, or it must be discarded.

6.0 Transportation of Chemicals

Below are some guidelines for transporting chemicals:

- Whenever chemicals are transported outside the laboratory, the container should be placed in a secondary, nonbreakable container.
- Carts should be used when possible.
- Before moving containers, check and tighten caps, taps, or other enclosures.
- Personnel at the destination area should be informed of the transport.

7.0 Chemical Waste Disposal

Disposing of wastes (including dyes and stains) through laboratory sinks and drains is absolutely prohibited at EPA laboratory facilities. All waste is handled through the EPA Waste Management Program. Only biohazardous and general laboratory waste is treated on-site. All waste regulated by the Resource Conservation and Recovery Act (RCRA) is sent off-site for recycling, fuel blending, or incineration.

All chemical waste generated at the EPA laboratory facilities must be submitted with the appropriate paperwork into the waste management program. The SHEMP

Manager will review the EPA Form 435 and any additional paperwork and will determine if the waste is RCRA-regulated or non-regulated waste. Based on this determination, the SHEMP Manager will make arrangements for appropriate disposal. Refer to Chapter C14 of this manual for specific guidelines on waste management.

Attachment F3-1: Incompatibility Table

Purpose: To provide a list of chemicals that could cause hazardous reactions due to incompatibility.

Instructions: Substances in the right-hand column should be stored and handled so they cannot possibly accidentally contact corresponding substances in the left-hand column. The following list contains some of the chemicals commonly found in laboratories, but it should not be considered complete.

Attachment F3-1: Incompatibility Table

Alkaline and alkaline earth metals, such as sodium, potassium, cesium, lithium, magnesium, calcium	Carbon dioxide, carbon tetrachloride and other chlorinated hydrocarbons, any free acid or halogen (Do not use water, foam or dry chemical on fires involving these metals)
Acetic annhydride	Chromic acid, nitric acid, hydroxyl-containing compounds, ethylene glycol, perchloric acid, peroxides, and permanganates
Acetone	Concentrated nitric and sulfuric acid mixture
Acetylene	Copper, silver, mercury, and halogens
Aluminum alkyls	Halogenated hydrocarbons, water
Ammonia (anhydrous)	Silver, mercury, chlorine, calcium hypochlorite, iodine, bromine, hydrogen fluoride, chlorine dioxide
Ammonium nitrate	Acids, metal powders, flammable liquids, chlorates, nitrates, sulfur, finely divided organics or combustibles
Aniline	Nitric acid, hydrogen peroxide
Benzoyl peroxide	Chloroform, organic materials
Bromine	Ammonia, acetylene, butadiene, butane and other petroleum gases, sodium carbide, turpentine, benzene, and finely divided materials
Calcium carbide	Water (see also acetylene)
Calcium hypochlorite	Methyl carbitol, phenol, glycerol, nitromethane, iron oxide, ammonia, activated carbon
Calcium oxide	Water
Carbon, activated	Calcium hypochlorite
Chlorates	Ammonium salts, acids, metal powders, sulfur, finely divided organics or combustibles
Chlorine	Ammonia, acetylene, butadiene, butane and other petroleum gases, hydrogen, sodium carbide, turpentine, benzene, and finely divided metals
Chlorine dioxide	Ammonia, methane, phosphine, and hydrogen sulfide
Chlorosulfonic acid	Organic materials, water, powdered metals
Chromic acid	Acetic acid, naphthalene, camphor, glycerine, turpentine, alcohol and other flammable liquids, paper or cellulose
Copper	Acetylene, hydrogen peroxide, ethylene oxide
Cumene hydroperoxide	Acids (organic or mineral**)**

Attachment F3-1: Incompatibility Table

Ethylene oxide	Acids, bases, copper, magnesium perchlorate
Fluorine	Almost all oxidizable substances
Hydrocyanic acid	Nitric acid, alkalis
Hydrogen peroxide	Copper, chromium, iron, most metals or their salts, any flammable liquid, combustible materials, aniline, nitromethane
Hydrides	Water, air, carbon dioxide, chlorinated hydrocarbons
Hydrofluoric acid, anhydrous (hydrogen fluoride)	Ammonia (anhydrous or aqueous), organic peroxides
Hydrogen sulfide	Fuming nitric acid, oxidizing gases
Hydrocarbons (benzene, butane, propane, gasoline, turpentine, etc.)	Fluoride, chlorine, bromine, chromic acid, sodium peroxide, fuming nitric acid
Hydroxylamine	Barium oxide, lead dioxide, phosphorus pentachloride and trichloride, zinc, potassium dichromate
Iodine	Acetylene, ammonia (anhydrous or aqueous)
Maleic anhydride	Sodium hydroxide, pyridine and other tertiary amines
Mercury	Acetylene, fulminic acid, ammonia, oxalic acid
Nitrates	Acids, metal powders, flammable liquids, chlorates, sulfur, finely divided organics or combustibles
Nitric acid (concentrated)	Acetic acid, aniline, chromic acid, hydrocyanic acid, hydrogen sulfide, flammable liquids, flammable gases, nitratable substances, organic peroxides, chlorates
Nitroparaffins	Inorganic bases
Oxygen	Oil, grease, hydrogen, flammable liquids, solids or gases
Oxalic acid	Silver, mercury, organic peroxides
Perchlorates	Acids
Perchloric acid	Acetic anhydride, bismuth and its alloys, alcohol, paper, wood, grease, oil, organic amines or antioxidants
Peroxides, organic	Acids (organic or mineral); avoid friction
Phosphorus (white)	Air, oxygen
Phospohorus pentoxide	Propargyl alcohol
Potassium chlorate	Acids (see also chlorates)
Potassium perchlorate	Acids (see also perchloric acid)

Attachment F3-1: Incompatibility Table

Potassium permanganate	Glycerine, ethylene glycol, benzaldehyde, any free acid
Silver	Acetylene, oxalic acid, tartaric acid, fulminic acid, ammonium compounds
Sodium	See alkaline metals
Sodium azide	Air, water
Sodium nitrite	Ammonium nitrate and other ammonium salts
Sodium oxide	Water, any free acid
Sodium peroxide	Any oxidizable substance, such as ethanol, methanol, glacial acetic acid, acetic anhydride, benzaldehyde, carbon disulfide, glycerine, ethylene glycol, ethyl acetate, methyl acetate and furfural
Sulfuric acid	Chlorates, perchlorates, permanganates, organic perioxides
UDMH	Oxidizing agents such as hydrogen peroxide and fuming nitric acid
Zirconium	Water, carbon tetrachloride, foam and dry chemical or zirconium fire extinguishers