

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



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A laboratory -specific SOP is required for <i>particularly hazardous substance (PHS)</i> and/or any operation involving a <u>High-Risk chemical</u> . PI approval of a lab specific SOP is required for all PHS and/or high-risk chemicals.	

1. DESCRIPTION:

Cryogenic liquids are gases that are kept in a liquid state at extremely low temperatures and are used in research to provide extremely low temperatures for frozen storage and experimentation.

Safety Data Sheets (SDS) use numbered hazard statements to communicate hazards, although the number may not always be present. The presence of the following hazard information indicates the chemical is a cryogenic liquid.

GHS Pictogram	TU Hazard Category	GHS Category	GHS H-Code	Signal Word
	Hazardous Chemical	Gases Under Pressure-Refrigerated liquified gas	H281	Warning

Contact EHRS via ehrschem@temple.edu for questions on the safe management of Cryogenic Liquids.

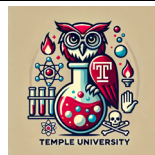
2. POTENTIAL HAZARDS:

The primary hazards associated with cryogenic liquids are frostbite/cryogenic burns/hypothermia, asphyxiation, fire/explosion, sudden release of pressure, and structural embrittlement.

- **Frostbite/Cryogenic Burns:** Cryogenic liquids and their associated cold vapors and gases can produce effects on the skin similar to a thermal burn. Brief exposures that would not affect skin on the face or hands can damage delicate tissues such as the eyes. Prolonged exposure of the skin or contact with cold surfaces can cause frostbite. Unprotected skin can stick to metal that is cooled by cryogenic liquids. The skin can then tear when pulled away.

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



- **Asphyxiation:** Cryogenic liquids have large liquid-to-gas expansion ratios and in confined or poorly ventilated areas the expanding gases will displace oxygen, presenting an asphyxiation hazard to personnel working in the area.
- **Fire/Explosion:** Oxygen has a higher boiling point (-183°C) than cryogenic liquids such as nitrogen (-195°C), hydrogen (-252.7°C), and helium (-269°C). As a result of this disparity, oxygen can be inadvertently condensed out of the atmosphere during the use of these lower-boiling cryogenic liquids, resulting in the creation of highly hazardous oxygen enriched atmosphere that can increase the risk of fire or explosion. Additionally, if a cryogenic liquid is subjected to a large amount of heat input, a flash vaporization can occur, resulting in a boiling liquid expanding vapor explosion (BLEVE). Liquid oxygen condensation in vacuum traps or from ice plug formation or lack of functioning vent valves in storage Dewars can also pose a serious explosion hazard.
- **Sudden Release of Pressure:** Sealed containers or closed off piping systems that contain cryogenic liquids can rupture when the cryogenic liquids are allowed to warm to room temperature. The rupture results as the warming cryogenic liquids change to the gas state and expand, producing enormous pressure within the container or piping system.
- **Structural Embrittlement:** Materials that are normally structurally sound, such as carbon steel, zinc, plastic, and rubber, can become brittle and fail due to thermal stress fracturing when subjected to cryogenic temperatures.

Check the Safety Data Sheet (SDS) to determine the specific hazard of the substance, including route of exposure and other hazard classifications, such as flammable, corrosive, etc. Follow other hazard specific guidelines as appropriate.

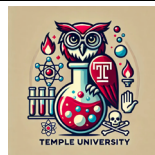
3. REQUIRED TRAININGS & INFORMATION:

Before beginning work with Cryogenic Liquids, all laboratory personnel must have completed the following:

- TU Required Trainings (available at [TU-EHSA](#))
 - Initial Safety Training for Researchers
 - Regulated Chemical Waste Management for Laboratories-Annual
- Cryogenic Liquids Safety Training
- Review the Temple University [Chemical Hygiene Program \(CHP\)](#)
- Review any required Laboratory-specific Standard Operating Procedures (SOPs)
- Review any relevant Safety Data Sheets (SDS) available from the manufacturer or from [TU-EHSA-Search for Safety Data Sheets](#)
- Review the online section on Laboratory Emergencies

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



- Complete any laboratory-specific training necessary to perform the procedures.
- Place signature on the Laboratory-Specific Training Checklist to indicate review.

4. HAZARD CONTROLS

Purchasing:

- When possible, look for alternative procedures or reagents that will eliminate the need for the cryogenic liquids, or substitute it for a less hazardous chemical.

Engineering Controls:

- Cryogenic liquids must only be used in well-ventilated areas designed to have 6-12 air changes per hour.
- Do not use cryogenic liquids in under-ventilated or non-ventilated areas such as cold rooms.
- Depending on the amount of cryogenic liquid in a room, the dimensions of the room, and the ventilation, an oxygen sensor may be necessary to alert personnel of an oxygen-deficient environment. Contact EHRS for assistance.

Administrative Controls-General:

- Never work alone. At least one other person familiar with the hazards of the gas must be present in the laboratory.
- Work with these chemicals must be performed in an area that is within 10 seconds (~ 55 feet) of a functioning safety shower and emergency eyewash.
- Verify and review your experimental setup and procedure before beginning work. Check that all equipment & piping/tubing used with cryogenic liquid is rated for use at cryogenic temperatures.
- Ensure all equipment is functioning properly.
- Inform your colleagues about the cryogenic liquid and where it will be used.
- Use the smallest necessary amount of the cryogenic liquid.
- Consult with your PI before scaling up procedures involving cryogenic liquids.
- If cryogenic liquids are to be used in research animals, ensure that they are listed on the laboratory's IACUC protocol.

Administrative Controls-Chemicals Categorized as a Particularly Hazardous Substance (PHS) or a "High-Risk" Chemical:

These chemicals require the following to reduce the risk of exposure to laboratory personnel:

- Development of a laboratory-specific Standard Operating procedure (SOP)

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



- Establishments of a designated area. A designated area may be the entire laboratory, an area of a laboratory, or a device such as a laboratory hood.
- Use of a containment device such as chemical fume hoods or glove boxes.
- Procedures on the safe removal of contaminated waste.
- Decontamination procedures.
- Restrictions on working alone with cryogenic liquids.
- Required PI approval before starting use.
- Special laboratory specific signs or labels indicating warnings or work practices to be followed.
 - A “High Hazard Substance Sign” must be posted in the designated area. Signs are available via EHRS.

Designated area locations, applicable work practices, and information regarding the general handling must be included in the laboratory-specific SOP and be communicated to laboratory personnel through laboratory specific training provided by the PI prior to beginning work.

5. PERSONAL PROTECTIVE EQUIPMENT (PPE)

The extreme cold and unique properties of cryogenic liquids make strict observance of the use of proper personal protective equipment (PPE) an absolute necessity. Failure to use proper PPE while working with cryogenics will result in severe injury or death. Follow the PPE recommendations listed in this guideline, Safety Data Sheets (SDS) and/or any required laboratory-specific SOPs for specific PPE recommendations. This should include at a minimum:

Skin and Body Protection:

Long pants (or equivalent) completely covering legs without cuffs, closed toed leather or safety shoes, and a traditional lab coat. The use of an apron made of non-woven material, such as leather, is also strongly recommended as a secondary form of protection when working with any more than just a few milliliters of cryogenic liquid. Short sleeved shirts and shorts offer no protection and must not be worn.

Hand Protection:

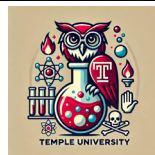
Gloves must be worn when handling cryogenic liquids. Hands must be protected with cryogenic gloves that fit loose enough to be quickly removed in case the liquid becomes entrapped close to the skin.

Eye Protection:

Eye protection in the form of ANSI Z87.1-compliant safety glasses with side shields and a face

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



shield must be always worn when handling cryogenic liquids.

Additional Hygiene Measures:

If hazardous chemicals come into contact with gloves, immediately dispose of the affected gloves as hazardous waste. If hazardous chemicals come into contact with reusable PPE (lab coat, etc.) immediately remove the affected PPE, then dispose of it or submit it for laundering.

Additional PPE may be required depending on the specific operations involving your experimental procedures.

6. STORAGE, HANDLING & TRANSPORTATION

Always follow the manufacturer's recommendations for storage and handling.

Storage:

Storage of cryogenic liquid cylinders or Dewars in hallways, unventilated closets, environmental rooms, and stairwells is prohibited. A cryogenic liquid storage unit left open to the atmosphere, or catastrophic failure of a storage unit, could create an oxygen deficient atmosphere. Follow these procedures to reduce the likelihood of this occurrence:

- Store Dewars in well-ventilated rooms with a minimum of six air changes per hour. Bulk cryogenic liquid dispensing areas within buildings must also be well-ventilated. EHRS recommends continuous oxygen monitoring equipment in bulk dispensing areas and may recommend detection systems and alarms for storage areas depending on location, quantity of material stored and ventilation rate. If the ventilation rate is unknown, contact EHRS for an evaluation.
- Dewars and cryogenic liquid cylinders should be placed so that vents and openings are oriented away from personnel and lab equipment.
- Do not store cryogenic liquids with corrosive or flammable chemicals.
- No more than one backup Dewar or cryogenic liquid cylinder is allowed per piece of equipment. Additional Dewars or cryogenic liquid cylinders must be stored in areas designed for such storage. Contact EHRS at ehrschem@temple.edu to evaluate potential storage locations.
- Glass Dewars must have an exterior coating/cover to minimize projectiles in the event of an explosion. Newer Dewars may have a plastic mesh over the exterior for this purpose. Older Dewars must be thoroughly taped or replaced.

Handling:

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



Cryogenic liquids have properties that make them more dangerous to use than other liquids: extremely cold temperatures, high liquid-to-vapor expansion ratios, and flammability for certain liquids. Contact with cryogenic liquids, cryogenic equipment, or splashing liquid can cause severe tissue damage. Burns, frostbite, tearing of the flesh, and eye damage are all possible injuries. Vapors from boiling liquids can also cause eye damage, frostbite to the skin, and oxygen deficient environments.

To minimize exposure during use, implement the following procedures:

- Wear appropriate PPE as discussed in PPE section.
- Do not wear jewelry or other materials that could trap liquid to the skin if spilled.
- Stay out of the vapor pathway.
- Ventilate the room in case of spill or leak.
- Use fume hoods when working with cryogenics if possible.
- Always use tongs when handling objects in cryogenic liquid.
- Only use approved materials with cryogenics. Unapproved materials (such as plastic, rubber, wrought iron, hollow tubes, and carbon steel) will become brittle and shatter or, in the case of hollow tubes, become over pressurized.
- Periodically inspect equipment, remove ice, and frost blockages from openings to prevent over pressurization.
- Do not tamper with pressure relief valves. Report any leaks or improperly set relief valves to the manufacturer.
- Do not use corrosive cleaning materials that could damage the metal jacket.

Dispensing and Transport of Cryogenic Liquids:

Special precautions must be taken to prevent a spill while dispensing or transporting cryogenics, in addition to minimizing exposures from liquids and vapors. The high liquid to vapor expansion ratio could rapidly displace all oxygen in a room and result in asphyxiation.

- Always wear proper PPE when dispensing or transferring cryogenic liquids.
- Use no fewer than two personnel to transport cryogenic liquids in large Dewars and cryogenic liquid cylinders; use handcarts equipped with brakes.
- Never transport cryogenic liquids on an elevator with live passengers. When transporting cryogenic liquids via elevator the sender should remain outside the elevator, push the button for the desired floor and let the doors close. Another person should be ready on the receiving floor to take the container off the elevator. All elevator doors from the starting floor to the floor of destination should be staffed to prevent entry by anyone between floors.
- Always use care when handling equipment. Damage to Dewars could result in the loss of vacuum in the jacket and increased evaporation.
- When carrying a Dewar, wear PPE and hold it as far away from the face as possible.

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



Containers that cannot be easily and safely carried should be placed on a stable wheeled base designed for the Dewar.

- When obtaining liquid from a large dispensing Dewar or cryogenic liquid cylinder, cool the secondary container by adding a little cryogenic liquid first. Then dispense slowly to mitigate thermal stress.
- Stay in constant attendance of the filling operation and do not overfill. Also, do not allow the cryogenic liquid to fall through a distance to reach the receiving vessel.
- When manually pouring liquid into a smaller dewar, ensure the secondary container is secured. Pour slowly to prevent excess splashing and do not overfill. Use a phase separator, if available, to control the vapor path while pouring.

Special Precautions for the Use of Cold Traps:

Cold traps are used when using instrumentation, a building vacuum system, water aspirator, or a vacuum pump. Cold traps prevent the introduction of liquids and vapors into and out of the system by providing a low temperature surface for molecules to condense. When using liquid nitrogen (LN₂) in cold traps, the following procedures must be implemented to prevent over pressurization and explosion:

Do not open the system to the atmosphere until the trap is removed.

In the event that the system is opened with the trap still in place, there is a possibility that oxygen will condense out of the air and combine with the organic material inside the trap.

There are two scenarios that could result: it could immediately create an explosive mixture and explode, or the oxygen could stay condensed in the liquid. **DO NOT RECLOSE THE SYSTEM.**

The condensed oxygen will vaporize after the trap is removed or the bath has evaporated resulting in an over pressurization and possible explosion.

Special Precautions for the Use of Cooling Baths:

Cooling baths are used to maintain low temperatures, typically between 13°C and -196°C.

These low temperatures are used to collect liquids after distillation, to remove solvents using a rotary evaporator, to perform a chemical reaction below room temperature, or to freeze cells. Cooling baths use ice, dry ice, or liquid nitrogen (LN₂) mixed with a solvent to achieve the low temperatures.

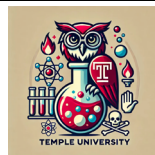
PPE for working with LN₂ cooling baths should include safety glasses, face shield, insulated heavy gloves, a buttoned lab coat, closed toe shoes, pants with no cuffs, and a long sleeve shirt.

Cool Dewar to mitigate stress.

Add a small amount of liquid nitrogen to Dewar, then add remaining LN₂, lastly add solvent to

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



LN₂.

Liquid nitrogen can create an asphyxiation hazard by displacing oxygen from the room. Work only in a well-ventilated area.

Always use tongs when handling objects in cooling bath.

Special Precautions for the Use of Cryotubes:

Cryogenic storage vials (cryotubes) are designed for vapor phase storage in the extremely cold nitrogen gas that sits just above the reservoir of liquid nitrogen in the bottom of the freezer or dewar. If the freezer/dewar is overfilled with liquid nitrogen and the cryotubes are immersed, leakage of liquid nitrogen into the cryotubes through minute cracks occurs. When the cryotubes are removed from the freezer/dewar, the liquid nitrogen expands rapidly as the cryotube thaws, resulting in explosions.

To avoid this problem do not overfill the freezer/dewar with liquid nitrogen and visually check each cryotube prior to filling to ensure there are no defects around the rim. Cryotubes should never be re-used.

As a precaution, gently remove vials from the dewar, holding the vial in the neck of the dewar for a moment before bringing them into room atmosphere. A tube that is going to explode will usually do so early in the warm-up process.

PPE for thawing cryotubes should include safety glasses, face shield, insulated heavy gloves, a buttoned lab coat, closed toe shoes and pants.

Cryotubes should be kept in a heavy, walled container or behind a safety shield while warming.

7. WASTE DISPOSAL PROCEDURES

All cryogenic cylinders **must** be returned to the gas supplier or properly disposed of as a *regulated chemical waste* through EHRS.

Accumulation Limits:

A maximum of one 2 empty, unused, expired and/or unwanted cryogenic cylinders may be accumulated in a laboratory (Satellite Accumulation Area (SAA)).

Specific Instructions:

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



1. Close and tighten valves and replace safety caps on cylinders.
2. Contact the vendor or supplier to obtain specific guidelines for shipment of cryogen cylinders to be returned to them.
3. If the cylinder cannot be returned to the vendor or supplier, contact EHRS to arrange for removal of cylinders. Refer to the section below for more instructions.
4. Write "Empty" or "MT" on the outside of each empty cylinder.
5. Cryogen must not be disposed of into sinks (the thermal shock is likely to damage the sink and/or the plumbing) or discarding in regular trash.
6. If a cryogen (such as dry ice) has been mixed with a liquid that would normally be disposed of as a regulated chemical waste, follow disposal procedures for that liquid).
7. Do not dispose of cryogenic liquids by pouring on the floor – this can result in damage to the floor and, in sufficient amounts, can cause an oxygen-deficient atmosphere.
8. Do not put unneeded cryogen in a sealed container, as the build-up of pressure could cause an explosion.

Removal:

1. Ensure that a properly completed "Hazardous Waste" tag is affixed to the cylinder. Tag must include the following:
 - a. Generator information is accurate.
 - b. Applicable Waste Stream is checked:
 - c. Applicable Hazards are identified: **Ignitable, Corrosive, Toxic or Reactive (as applicable)**
 - d. All chemical constituents and amounts (%) are included on the tag.
2. Submit a Chemical Waste Pickup Request Form prior to exceeding the accumulation limits.
3. EHRS will typically process a completed and accurate waste request within 3 days.

Contact EHRS via ehrschem@temple.edu for tags, collection containers and for any questions regarding proper waste disposal. Refer to Chemical Waste Management Program (CWMP) for more information.

8. EMERGENCIES

In the event of an emergency – Call Temple University Public Safety (TUPS) at (215) 214-1234 & EHRS at (215) 707-2520.

Health-Threatening Emergencies

1. Contact TUPS at 1-12234 from a campus landline phone or 215-203-1234 from a cell phone.
2. Go to the Temple University Hospital or nearest emergency room. Bring a copy of the SDS

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



for the chemical.

Temple University Hospital Emergency Department
Broad & Ontario Streets
Philadelphia, PA 19140

3. Notify your PI and Temple University-Human Resources-Absence Management
4. Notify EHRS at 215-707-2520 or eh@temple.edu as soon as possible, but within 8 hours of the incident.

Non-Health-Threatening Emergencies

1. Notify your PI and Temple University-Human Resources-Absence Management.
2. If necessary, go to Temple University Employee Health Services. Bring a copy of the SDS for the chemical.

Temple University Employee Services on Main Campus
1700 N. Broad Street, 4th floor
Philadelphia, PA 19122
Tel:215-204-2679

Additional Instructions:

Skin Contacts

Rinse body thoroughly using a safety shower for at least 15 minutes. If feasible, remove any contaminated or potentially contaminated clothing and/or jewelry.

Eye Contact

Rinse eyes thoroughly using an eyewash station for at least 15 minutes, occasionally lifting up the upper and lower eyelids to allow rinsing of the area beneath the eyelids. Remove contact lenses if possible.

Ingestion of chemical

Do not induce vomiting unless directed otherwise by the SDS or Poison Control (800-222-1222), Rinse mouth with water.

Inhalation of chemical

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



Move into fresh air.

Needle stick/puncture exposure.

Wash the affected area with soap and water for 15 minutes. Contact Poison Control 800-222-1222

Fires:

If a small fire occurs, you are trained in the use of fire extinguishers, and you feel safe doing so, use a fire extinguisher to put out the fire. If a single extinguisher cannot put out the fire, activate a fire alarm and evacuate the area. Wait for emergency responders to arrive so that you can provide information about the fire.

Refer to Laboratory Emergencies for additional information.

9. SPILL RESPONSE

1. Assess the extent of the gas release and its hazards. DO NOT enter the area if you cannot assess the conditions of the environment well enough to be sure of your own safety.
2. If the gas release poses a chemical hazard (due to toxicity or reactivity) or if a significant amount of gas was released into the room, evacuate the room.
3. ***Immediately contact TUPS at 1-1234 or 215-204-1234.***
4. If safe to do so, help contaminated or injured persons evacuate.
5. Keep others from entering the area (e.g., use caution tape, barriers, etc.).
6. Stay away from the spill area and give the building ventilation time to dissipate the gas.
7. Report the spill to EHRS at 215-707-2520 or ehrs@temple.edu if you are not sure if the atmosphere is safe to enter.

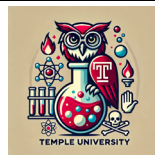
NOTE: With cryogenic liquids, even a relatively small spill can create an oxygen-deficient atmosphere, as all cryogenic liquids expand in volume significantly upon conversion to gas. As a reminder, a 1 L spill of liquid nitrogen will evaporate into roughly 700 liters of pure nitrogen gas.

Minor Spill

If the amount and type of chemical spilled presents no potential for a hazardous chemical exposure and if you are trained to do so, wear appropriate PPE, and clean up the spill. Double bag any contaminated cleaning materials and manage as a regulated chemical waste. Contact EHRS at 2-2520 to report the spill if you are not trained and/or there is a risk of chemical

CHEMICAL SAFETY GUIDE

CRYOGENIC LIQUIDS



exposure.

Major Spill

Defined as any hazardous chemical spill that:

- would result in chemical exposure during cleanup,
- due to its size and/or hazards, requires capabilities beyond your training, or
- results in a release into the environment (e.g., a spill goes down a drain).

Call TUPS at 215-204-1234 from a cell phone or 1-1234 from a campus phone for assistance.

Refer to Laboratory Emergencies for additional information.

10. REFERENCES

1. Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards.
National Research Council, Updated Version-2011
2. 29 CFR 1910.1250-Occupational Exposure to hazardous chemicals in laboratories.
3. Temple University Chemical Hygiene Plan