Compressed Liquid Gases (Cryogens)

Laboratory Safe Work Practices


Compressed Liquid Gases (Cryogens)

Cryogenic liquids are liquefied gases that have a normal boiling point below -238°F (-150°C). Liquid nitrogen is used in the microbiology laboratory to freeze and preserve cells and virus stocks. The electron microscopy laboratory, frozen section suites, and grossing stations for surgical pathology frequently use liquid nitrogen; some laboratories also use liquid helium. The principal hazards associated with handling cryogenic fluids include cold contact burns and freezing, asphyxiation, explosion and material embrittlement.

Cold contact burns and freezing

- Liquid nitrogen is dangerously cold (-320°F [-196°C]), and skin contact with either the liquid or gas phase can immediately cause frostbite. At -450°F (-268°C), liquid helium is dangerous and cold enough to solidify atmospheric air.
- Always wear eye protection (face shield over safety goggles). The eyes are extremely sensitive to freezing, and liquid nitrogen or liquid nitrogen vapors can cause eye damage.
- Do not allow any unprotected skin to contact uninsulated piping, hoses, tongs, spargers, specimen box storage racks, or other metal objects because these become extremely cold when exposed to liquid nitrogen. Skin will stick to the metal, tearing the flesh when one attempts to withdraw from it.
- When filling cryogenic dewars, wear long-sleeved shirts or laboratory coats, long trousers (preferably without cuffs which could trap the liquid), closed shoes (never sandals or open shoes), and insulated cryogloves labeled as appropriate for use with cryogenic liquids. Do not tuck pant legs into shoes or boots; doing so could direct liquid into the foot coverings and trap the cryogenic liquid against the skin.
- Wear loose-fitting thermal gloves with elbow-length cuffs when filling dewars. Ensure that gloves are loose enough to be thrown off quickly if they contact the liquid.
- Never place gloved hands into liquid nitrogen or into the liquid nitrogen stream when filling dewars. Gloves are not rated for this type of exposure. Insulated gloves are designed to provide short-term protection during handling of hoses or dispensers and during incidental contact with the liquid. Use special cryogenic liquid tongs when retrieving items from liquid nitrogen.
- Liquid nitrogen confers a high risk of splattering; jets of liquid nitrogen can be generated when canes, canisters, and other objects that are at much higher temperatures are placed into liquid nitrogen. These activities can present a freezing hazard.
- Do not insert a hollow tube into the liquid nitrogen because liquefied gas may spurt from the tube.

Asphyxiation hazards

- Although nitrogen is nontoxic and inert, it can act as an asphyxiant by displacing the oxygen in the air to levels below that required to support life. Inhalation of nitrogen in excessive amounts can cause dizziness, nausea, vomiting, loss of consciousness, and death without warning.
- When liquid cryogens are expelled into the atmosphere at room temperature, they evaporate and expand to 700–800 times their liquid volume. Even small amounts of liquid can displace large amounts of oxygen gas and decrease the oxygen content of the atmosphere below a safe level (23,38,42,43).
- Do not store dewars or nitrogen containers in a confined space. The venting gas could displace enough oxygen to become a hazard.
- If enclosed spaces must be used, install oxygen monitors. Train personnel to leave the area immediately if the alarm sounds. The alarm must be audible both inside and outside the room to prevent anyone from entering the room.
Explosion hazards

- Liquid gases, even those considered inert, can present explosion hazards.
- Heat flux into the cryogen is unavoidable regardless of insulation quality. Cryogenic fluids have small latent heats and will expand 700–800 times as they warm to room temperature. Therefore, even a small heat input can create large pressure increases within the vessel.
- Dewars must be moved carefully. Sloshing liquid into warmer regions of the container can cause sharp pressure rises.
- Do not drop, tip, or roll containers on their sides; doing so could damage the vessel and/or cause a sharp increase in internal pressure.
- Cryogenic containers are equipped with pressure relief devices designed to control the internal pressure. Cryogenic containers will periodically vent gases. This is normal. Do not plug, remove, or tamper with any pressure relief device.
- Vents must be protected against icing and plugging. When all vents are closed, the expanding gas can cause an explosion. Vents must be maintained open at all times.
- Always use special ultralow-temperature containers to hold liquid nitrogen. Never place liquid nitrogen into domestic thermos flasks because they are not designed to withstand the large and rapid temperature changes that occur when liquid nitrogen is placed in the vessel (42,43).
- Fill liquid nitrogen dewars slowly to minimize the internal stresses of cooling. Excessive stress could damage the vessel and cause it to fail.
- Liquid helium is cold enough to solidify atmospheric air. Only helium is to be introduced or allowed to enter the helium volume of a liquid helium dewar. Take precautions to prevent air from back-diffusing into the helium volume.
- Liquid nitrogen and liquid helium have boiling points below that of liquid oxygen, and they can condense oxygen from the atmosphere. Repeated replenishment of the system can cause oxygen to accumulate as an unwanted contaminant. Similar oxygen enrichment can occur where condensed air accumulates on the exterior of cryogenic piping. An explosion could occur if this oxygen-rich liquid is allowed to soak insulating or other materials that are not compatible with oxygen. In addition, some oils can form an explosive mixture when combined with liquid oxygen.

Cryotube explosions

- PPE includes an ANSI-specification, impact-resistant face shield, heavy gloves, and a buttoned laboratory coat during removal of cryotubes and ampoules from nitrogen tanks.
- Whenever possible, store ampoules in the gaseous phase rather than submerging in the liquid nitrogen of the cryogenic dewar. An imperfectly sealed ampoule will pick up less nitrogen in the gaseous phase.
- Cryotubes and glass ampoules used for freezing cells and viruses can explode without warning when removed from cryogenic storage. These tube explosions are presumed to be caused by entry of liquid nitrogen into the tube through minute cracks; as the tube thaws, the rapidly expanding gas causes the tube to explode, scattering the contents of the tube (23).
- Nitrogen outgassing from an imperfectly sealed vial will sometimes produce a hissing noise before the vial explodes. The absence of hissing does not mean the vial is safe. Place cryotubes and ampoules onto gauze or paper toweling in an autoclavable, heavy-walled container immediately after removal from the nitrogen tank and close the lid of the heavy-walled container quickly. If an explosion occurs, autoclave the entire vessel.

Embrittlement

- Never pour cryogenic liquids down the drain. Laboratory plumbing is one of many ordinary materials that become brittle at cryogenic temperatures and easily fracture.
- Wood and other porous materials may trap oxygen at low temperatures and will explode when subjected to mechanical shock (42).

Infectious disease hazards

- Liquid nitrogen can become contaminated when ampoules are broken in the dewar, and contaminants can be preserved in the nitrogen (23). These potentially infectious contaminants can contaminate other vials in the dewar and generate an infectious aerosol as the liquid nitrogen evaporates.
- Plastic cryotubes rated for liquid nitrogen temperatures are recommended for liquid nitrogen storage because they appear to be sturdier than glass ampoules and are less likely to break in the nitrogen.