GATEWAY UNIVERSITY RESEARCH PARK
Liquid Nitrogen Safety

Liquid Nitrogen Safety

STATEMENT OF UNDERSTANDING AND COMPLIANCE

I confirm that I have read and understand the Liquid Nitrogen Safety Guidelines and will comply with the procedures and policies.

Name/Title **Signature** **Date**

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Liquid Nitrogen & Cryogenic Liquids

Liquid nitrogen, LN2, is colorless, odorless and tasteless. It is non-flammable, physiologically inert and non-toxic. It is an extremely cold liquid with a boiling point of -196ºC = -320ºF.

Cryogenic liquids are liquefied gases that are kept in their liquid state at extremely low temperatures. Cryogenic liquids have boiling points below -150°C (-238°F) (Carbon dioxide and nitrous oxide, which have slightly higher boiling points are sometimes included in this category). All cryogenic liquids are gases at normal temperatures and pressures. All Cryogenic liquids produce large amounts of gas when they vaporize.

There a many applications of LN2 in a biomedical research facility, one of the most common being cryopreservation, the storage of cells at temperatures below -130 ºC, is essential in order to preserve biological material unaltered.

Safety Hazards Associated with Handling LN2

- The extremely low temperature of the liquid can cause **severe frostbite or eye damage** upon contact. *Symptoms of frostbite include change in skin color to white or grayish yellow and the pain after contact with* LN2 *may quickly subside*. Items in contact with liquid nitrogen become extremely cold. Touching these items may result in torn flesh. Many substances become brittle upon contact with liquid nitrogen and may shatter when cold (such as common glass and large solid plastics), sending pieces of the material flying.
- On vaporization it expands by a factor of 700; one liter of liquid nitrogen becomes 24.6 cubic feet of nitrogen gas. This can cause **explosion** of a sealed or insufficiently vented container.
- Because the boiling point of oxygen is above that of nitrogen, oxygen can condense from the air into the liquid nitrogen. If Dewars and insulated flasks containing liquid nitrogen are left uncovered for an extended period of time, liquid oxygen can build up to levels which may cause **violent reactions** with organic materials (i.e. a severe clothing fire could result).
- Liquid nitrogen gas released in a confined space can displace sufficient oxygen to make the atmosphere incapable of sustaining life and cause **asphyxiation (suffocation)** without warning. Entering an oxygen deficient atmosphere may cause unconsciousness without warning and death without regaining consciousness. Symptoms of asphyxiation may include but are not limited to:
    - Feeling of tightness or compression in the head (near forehead)
    - Tingling sensation in the tongue, fingertips and toes
    - Difficulty and weakening of speech, leading to the inability to utter a sound
    - Unnoticeable and then rapid reduction of the ability to exert physical effort and to coordinate movements, leading to total immobility
    - Reduced awareness of the outside world and damping of sensory characteristics

- **It should not be presumed that any of the symptoms listed above would be felt or detected; you will not have sufficient warning.**

Requirements for Use of LN2

- It is the Principle Investigator's or Supervisor's responsibility to ensure that students/staff handling liquefied gases are instructed and trained as to the nature and the safe handling of LN2.
- Researchers must complete GURP General Lab Safety Training and must be in compliance with this LN2 Safety Plan.
- Researchers shall wear the required personal protective equipment.
- **Reporting Accidents Associated with Handling and Transporting LN**2**:** All accidents, e.g. spills and burns, must be reported to GURP EHS Manager.

Personal Protective Equipment (PPE) Required When Handling LN2

- Face shield required. *LN2 can splatter, possibly onto face and eyes. Safety glasses (even with side shields) or goggles are not sufficient to protect the face.*
- Insulated gloves (gloves should be loose fitting, so they can be quickly removed if liquid pours into them, or they should be elastic cuff insulated gloves).
- A splash resistant lab coat is required to minimize skin contact. A floor length splash resistant apron may also be worn.
- Closed toe shoes that cover the top of the foot or boots with cuff-less trouser legs extended over the top of the boots to prevent LN2 from spilling into them.

Rules and Precautions for Handling/Transporting/Storing LN2

- Use LN2 only in well-ventilated areas.
- Never dispose of LN2 by pouring it on the floor or into a sink. It could displace enough oxygen and cause suffocation and cause damage to materials it contacts. (Nitrogen is colorless and odorless – the cloud that forms when you pour LN2 is condensed water vapor from the air, not nitrogen gas.)
- Do not allow any LN2 to touch any part of your body or become trapped in clothing near the skin.
- Do not touch any item that has been immersed in LN2 until it has warmed to room temperature.
- Never dip a hollow tube into LN2; it may spurt liquid.
- Metals to be used for equipment in LN2 must possess satisfactory physical properties at the low operating temperatures.
- Introduction of a substance which is at normal room temperature into a cryogenic liquid or gas is always somewhat hazardous. There is a violent evolution of gas, and there is likely to be considerable splashing of liquid.

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Cryogenic Liquid Containers

- Use only suitable vessels for the handling and/or transport of cryogenic liquids. Do not store LN2 in any container with a tight fitting lid; a loose fitting lid helps in preventing air or moisture from entering the container and at the same time allows pressure to escape. A tightly sealed container will build up pressure as the liquid boils and may explode after a short time. Do not store LN2 for long periods in an uncovered container.
- **Dewars** are non-pressurized, vacuum-jacketed vessels, having a loose fitting plug or cap to prevent air and moisture from entering. At the same time, such flasks allow excess pressure to vent. Many labs use these to transport small amounts of LN2 or to keep cryogenic samples.
- **Cryogenic Liquid Cylinders or VGLs** (vent/gas/liquid) are portable vacuum-insulated pressurized containers, specifically designed for cryogenic liquids. These cylinders have valves for filling and dispensing the cryogenic liquid. As an additional protection these cylinders also have pressure-control valves with a bursting disk. These cylinders are well insulated but at times the extremely low temperatures of the cryogenic liquids can lead to constant heat leak and vaporization. The cryogenic product when not used will lead to pressure in the cylinder and often vent via the container's pressure relief device. However, this is a normal and safe function of the cylinder.
- Do not store containers in areas where they have a chance to come in contact with moisture and keep away from sources of heat.
- Store all cryogenic liquid containers in well-ventilated areas and these containers will vent to relieve any pressure buildup.

Storage of VGLs

- VGLs are generally delivered by Airgas National Welders-Specialty Gas Division to researchers at GURP.
- Ensure that all incoming containers are not damaged before storing.
- VGLs received from Airgas National Welders come in a variety of types: they may be low pressure VGLs with a pressure of ~22 PSI or high pressure VGLs with a pressure of ~350 PSI; they may be welded onto square wheeled bases with handles; free standing cylinders within a cradle trolley; or non-mobile free standing VGLs delivered by Airgas National Welders with a dolly.
- VGLs are very substantial in weight whether full or empty.
- Compressed gas cylinders are high pressure vessels, generally 1200 PSI (CO_2) to 2400 PSI and therefore these cylinders are required to be secured when stored. There are <u>no</u> requirements to secure the free- standing LN2 VGLs, though it would be good practice to secure these if possible. If securing the VGLs is not possible, store these in areas away from traffic, preferably against a wall or in a nook, on level ground, and prevent items from falling onto the VGLs or impacting the VGLs.

Transport

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- ONLY the freight elevator shall be used for transport of LN2. Passengers shall not ride in the elevator with any container of LN2. Make arrangements for someone to send the elevator to a receiving person waiting on the desired floor. In the event the freight elevator is not available, contact GURP Facility Services Director.
- Handle liquid cylinders carefully; do not roll, drop or tip them on their sides.

Filling Containers with LN2 or Transferring LN2

- Never leave the vessels unattended while transferring LN2. Ensure that the delivery of the LN2 is directly below the mouth of the receiving vessel; do not let the LN2 travel through a distance to reach the receiving vessel.
- Always fill warm Dewars slowly to reduce temperature shock effects and to minimize splashing.
- Always make sure that containers of LN2 are suitably vented and unlikely to become blocked due to ice formation. Never cork or plug the outlet to such containers.
- Do not fill cylinders and Dewars to more than 80% of its capacity.
- Protect floors while filing Dewars with cardboard, a floor saver or barrier matting (contact Facility Services Director). Repairs to fix damage to floors from liquid nitrogen spills will be paid by the PI/Department.

Storage of Cryovials

- Do not store cryovials in the liquid phase of LN2 unless specifically approved by the manufacturer for -liquid phase storage. Liquid can still enter closed screw-top cryovials with O-rings and explode when removed from storage because of the expansion factor.

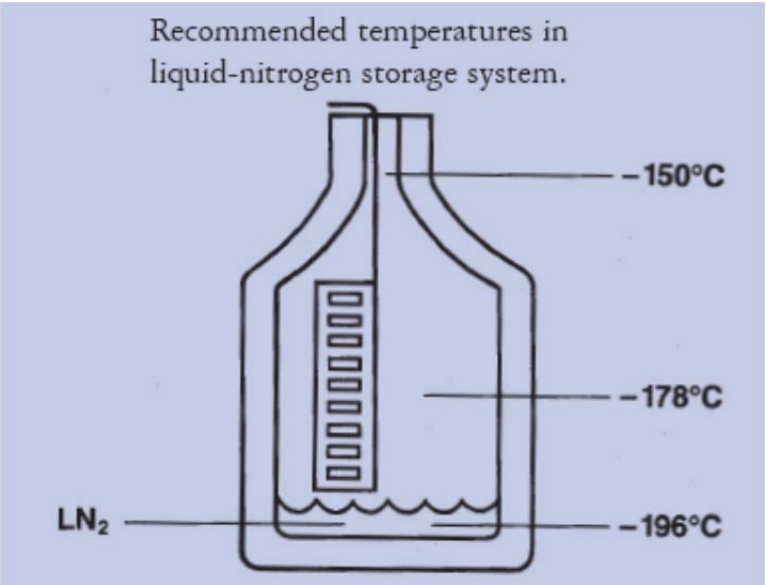

Figure from NuncTM Cryopreservation Manual.

- Laboratory personnel must use extreme caution when preserving samples in LN2. LN2 storage consists of a liquid phase and a gaseous phase. If cryovials are immersed in the liquid phase, LN2 can still enter the closed screw-top cryovials with O-rings during storage. The cryovial may then explode when it is removed from storage due to the vaporization and expansion (700x expansion ratio) of the liquid nitrogen inside the cryovial.
- Use only manufacturer-approved containers (e.g., cryovials) for storage in LN2.
- If storage in the LN2 liquid phase is required, utilize either:
    - Manufacturer-approved cryovials specifically designed for liquid phase storage; or
    - Gaseous phase-approved screw-top cryovials that are then hermetically sealed in an outer protective envelope designed for use in LN2. Nalge Nunc International manufactures CryoFlex™ tubing specifically for hermetically sealing cryovials for liquid phase storage.
    - Where feasible, the risk of explosion of cryovials stored in the LN2 liquid phase can be further reduced by moving cryovials to the gaseous phase in the LN2 container for at least 24 hours before removing.
    - Where feasible, the handling of containers (e.g., cryovials) inside of Biological Safety Cabinets may further reduce the risk of injury from explosions caused by excess pressure within containers.

First Aid

In the event a person is injured by LN2, the following first aid treatment should be given pending the care of a physician **only** if there is no risk to you:

- If any LN2 *or "cold boil off"* contacts the skin or eyes, immediately flood that area of the body with large quantities of unheated *(warm or tepid*) water and then protect frozen parts with loose, bulky, dry sterile dressings. ***DO NOT USE HOT WATER!***
- Calm the victim and avoid aggravating the injury. People with frostbitten feet should not walk on them. Do not rub or massage the affected parts of the body.
- If the skin is blistered or there is any chance that the eyes have been affected, get the patient immediately to a physician for treatment. ***Take a copy of the MSDS to the physician.***

References

Air Products Safetygram-7: Liquid Nitrogen
Air Products Safetygram-27: Cryogenic Liquid Containers
NuncTM Cryopreservation Manual