# Use of Liquid Nitrogen ($LN_2$)

## Introduction

The aim of this guidance is to give basic information on the use of Liquid Nitrogen ($LN_2$). You should consult your line manager / supervisor about the specific use of $LN_2$ in your area. It is important to be aware of the risks $LN_2$ can pose as fatalities have occurred.

The most common use is for cryopreservation of cells and microbes. It is also used for snap freezing of tissue samples prior to cryostat sectioning or other analysis.

The major problems associated with cryogenic gases are burns and asphyxiation due to Oxygen depletion. In some rare cases Oxygen enrichment may also be a problem.

## Physical Properties

The following properties are based on a standard atmosphere and temperature. The expansion ratio of $LN_2$ may change depending on the ambient temperature and pressure.

| | |
|---|---|
| Liquid Nitrogen | $LN_2$ |
| Colourless | |
| Odourless | |
| Molecular Weight | 28 |
| Boiling Point | -196 °C. |
| Vapour Density | 0.97 (Air=1) Due to its low temperature it will accumulate from the floor upwards. |
| Expansion Ratio liquid to gas | 1:683 |

## *Hazards associated with Liquid Nitrogen ($LN_2$)*

### 1. Cryoburns

Contact with $LN_2$ will result in cryoburns. These will be similar to severe frostbite and depending on the skin damage caused, could result in the amputation of affected digits or severe skin damage that may require plastic surgery. Blindness could occur if Liquid Nitrogen is splashed into the eyes.

Burns can occur in three ways:

*A.* *During direct contact with Liquid Nitrogen*

Under no circumstances should items be retrieved from dewars or other storage vessels using gloves or bare hands. Liquid Nitrogen will pass through all types of cryogloves. Limbs and other body parts must be kept out of fluid streams.

*B.* *Discharge of Liquid Nitrogen leading to splashes*

This may be either through a leak, accidental spillage, a flask being knocked over during transit, during refilling of a flask, and splashes or splattering occurring during the refilling of dewars or the retrieval of items.

*C.* *Contact of skin with super-cooled items*

This may occur when cryotubes are handled with bare hands. It can also occur when super-cooled metals such as pipes are handled. In the worst case this can lead to the loss of skin, frost bite or large scale tissue damage or skin, frost bite or large scale tissue damage.

## 2. Oxygen Depletion

Oxygen depletion can be caused by the steady evaporation of stored Liquid Nitrogen. It can also be caused by the sudden release of gas from a dewar, storage tank or pressurised vessel.

One litre of Liquid Nitrogen will form up to 694 litres of gas depending on the temperature and pressure. An average sized 25L Dewar contains the equivalent of 17,350 litres of free gas while a 250 litre Dewar contains the equivalent of 173,500 litres of free gas. The following calculation shows how the release of liquid nitrogen can cause severe Oxygen depletion in a room.

RV = Room Volume. LNV = Liquid Nitrogen volume in

Dewar * 694 $\%O_2$= 0.2095 Nitrogen volume (LNV-RV) / RV.

The density and temperature of Liquid Nitrogen will cause a room to fill with gas from the floor upwards should a leak occur.

### Effects of Oxygen depletion

Exposure to atmospheres containing less than 19.5% Oxygen can have a variety of effects. It should be borne in mind that different people will react in different ways when the local Oxygen concentration falls below <19%.

| % Atmospheric Oxygen | Effects |
|---|---|
| <19.5% | Unable to undertake physical activity for long periods. The colour of lips and skin may change in some individuals. Some people may also suffer from increased heart rate and respiratory problems. |
| <15% | Mental impairment, poor coordination increased breathing |
| <10% | Unconsciousness, blue lips, change of skin colour mental |
| <6% | Coma usually within 40 seconds |

### Oxygen Enrichment

The low temperature of cryogenic liquids can cause the local concentration of atmospheric oxygen to increase as other gases condense out from the atmosphere. This generally happens in the vicinity of super-cooled pipe work where blue streamers of Oxygen gas can sometimes be observed. Care should be taken when switching on electrical equipment that is not rated as spark free as it could potentially start a fire.

### Embrittlement of materials

Many soft materials such as plastic, rubber, lab flooring, etc. can be brittle after contact with $LN_2$ and they can easily shatter. The affected the materials will also be weakened and not support the usual load.

Disposal of $LN_2$ via sinks can lead to the plumbing becoming fractured.

# *Working with Liquid Nitrogen*

Only trained individuals should handle $LN_2$. The training must be documented.

Lone working with $LN_2$ should be avoided if possible and people should be notified if you intend to enter the basement cryostore or the CL3 service corridor.

## 1. Dewars and pressure vessels

Liquid Nitrogen and other cryogenic liquids such as Liquid Helium, Liquid Hydrogen and Liquid Argon are commonly stored in dewars. Dewars are a glass or metal container made like a vacuum bottle. They are a larger are more high-tech version of thermal mugs.

Dewars can unpressurised or can be pressurised depending on the volume and temperature of the cryogenic gas they will be holding. The largest pressurised dewars used within SGUL hold 250L of $LN_2$.

All dewars must be visibly inspected for cracks in the neck area when they are used. The polystyrene insert and the lid should always be kept fully closed when the dewar is not in use.

Pressure vessels should be checked for venting gas each time they are used. Valve connections must not be sealed with PTFE tape if venting occurs. The manufacturer should be contacted to arrange a service as soon as possible.

Avoid transporting pressure vessels or dewars over rough ground as this could cause cracks to develop in the flask with consequent loss of liquid.

## 2. Dewars and Cell Stores

Mammalian cells, stem cells, microorganisms including viruses, plant cells, embryonic tissues and other biological items are commonly stored in either the gas or liquid phase of $LN_2$

Care should be taking when filling small Dewars due to the potential for Liquid Nitrogen splashes to occur. Care should also be taken when placing vials into storage as the temperature difference between the vial and the liquid can result in splashes occurring.

Vials that have been retrieved from $LN_2$ storage should not be held at or near face level directly after removal from Dewars as they may shatter due to the material being unable to withstand the change in temperature or explode due to the sudden rise in temperature causing an increase in the pressure within the tube.

When removing vials from cell stores, a full face shield should always be worn. This reduces the likelihood of a penetrating eye injury in the event that a cyrotube explodes / shatters due to the change in temperature. The vial should always be handled while wearing cryogloves - nitrile, PVA, PVC or other gloves will lose their flexibility on exposure to low temperatures.

## 3. Confined Spaces and Lifts

Liquefied Nitrogen forms a vapour that while lighter than air will accumulate from the floor upwards due to its low temperature and can accumulate in confined spaces. If entry into an area where large amounts of liquid gas are stored is required, it would be advisable to notify a colleague that entry is taking place.

Never enter an area where the Oxygen monitoring alarm is sounding. Never override the alarm – always contact a lab manager or the SHE office for advice.

NEVER GET INTO A LIFT WITH ANY LIQUEFIED GAS VESSEL. SHOULD A FAILURE OF THE VESSEL OCCUR THIS WILL RESULT IN OXYGEN DEPLETION AND COULD PROVE FATAL.

## 4. Liquid Nitrogen Storage facility

The main Liquid Nitrogen store is based in the basement of Jenner Wing in room 01.234. The room is equipped with storage vessels connected to an automatic fill system as well as dewars.

There are four Oxygen depletion monitors to cover different zones and there are high volume fans to increase the ventilation in the event of oxygen depletion.

It is inadvisable to enter the room when the alarms are sounding. If all the alarms are sounding and the ventilation motors are not running contact the laboratory managers and the SHE Office immediately and do not enter the room.

The main storage tank is located outside Jenner Wing in a mesh cage and contains 4000L of Liquid Nitrogen. Should liquid be observed to be leaking from the tank, evacuate the area immediately and contact the SHE Office and the Laboratory Managers immediately

## 5. CL3 Service Corridor

The CL3 service corridor on the second floor contains two 250L pressurised dewars. The corridor has Oxygen depletion monitors fitted. Access to this area is restricted to members of the core support staff, and specialist staff only. Never enter the area if the alarm is sounding.

## 6. Transporting $LN_2$

It is important to avoid handling Dewars roughly as this could lead to the breakage of the internal lining and the loss of the thermal insulation. Never drag a dewar across the floor.

It is advisable for two individuals to work together to move the 25L Dewars on roller bases. It is important to be aware that the floors are not always smooth and the potential for the roller base to catch on manhole covers.

If lifts are used to move 25L or larger Dewars, the lift should be sent to the required floor and persons stationed at each level to prevent people entering the lift. The protective chain must be placed across the entrance to prevent accidental entry to the lift from staff on other floors. It is important that the roller base of the dewar is a suitable for and that the dewar does not slide about on the base. Passenger lifts should NEVER be used to transport $LN_2$ dewars or pressure vessels.

Smaller portable stainless steel dewars can be taken up the stairs (containing a maximum of 1 L $LN_2$). It is important that the lids are secured properly.

# *Personal Protective Equipment*

## 1. Cryogloves

Cryogloves and aprons can either be made from leather or hide or from a thermal mixture or lined with Thinsulate. It is best to obtain gloves and aprons that are as tightly fitting as possible to prevent liquid coming into contact with the skin. Neither type of glove should be exposed directly to Liquid Nitrogen or other cryogenic liquids. The blue type of cryogloves or aprons should not be used in wet environments as they are not waterproof. It is advisable to use gloves made to the EN 511 standard.

## 2. Footwear

Suitable footwear must be worn when handling Liquid Nitrogen as even brief contact can lead to frostbite.

## 3. Eye protection

A visor or face shield must be worn when filling or decanting cryogenic liquids to avoid impact on the eyes or face. A shield or visor should also when removing cryotubes from storage. The selected face shield must be capable of both withstanding splashes of $LN_2$ and impacts from exploding / shattering cryotubes. It is advisable to use a shield made to the EN166 standard.

## 4. Clothing

It is advisable to wear a lab coat when accessing the dewars and cell stores to reduce the likelihood of $LN_2$ being splashed onto clothing.

It is advisable to wear a cryoapron when filling the dewars in the store to reduce the likelihood of $LN_2$ being splashed onto the torso while filling the dewars.

# *Disposal of Liquid Nitrogen*

Excess Liquid Nitrogen should be allowed to evaporate preferably in an area with a high ventilation rate from which people can be excluded.

Liquid Nitrogen ***must never*** be poured onto a floor or down a laboratory sink as the $LN_2$ can lead to embrittlement and damage of the material.

# *Treatment of Cold burns*

***Skin***

Depending on the volume of $LN_2$ that has been splashed onto the skin, damage may not be immediately visible although an individual may feel a slight tingling. If the skin appears waxy, this is an indication that damage has occurred.

The skin should be slowly thawed with cool or lukewarm water. Use of hot water will cause rapid thawing that could cause a more severe injury.

If the area feels numb a first aider should be called and the individual should be taken to the Accident and Emergency department in St. James Wing.
The affected area should be loosely covered with a sterile dressing to prevent further injury.

***Eyes***

If $LN_2$ has been splashed onto the eyes, call for help and ask the person to try to irrigate the eye with water at room temperature.

A first aider should be summoned (ext 0909).

The injured person should go as soon as possible to Accident and Emergency in St. James Wing.

It is important to inform the triage nurse that the eyes of been splashed so the injured person can be rapidly treated.

# *Asphyxiation*

Do not attempt to rescue anyone in a room where an Oxygen alarm is sounding and the person appears to be unconscious. The lab managers (1603 / 5400) or the SHE office (0637 / 5166 / 5365) should be contacted immediately. If there is time summon a first aider and ask them to contact individuals who have been trained to use self-contained breathing apparatus.

If an alarm is sounding and the person is awake but displaying the following behaviours

- Changes in colour

- Rapid breathing
- Disorientation
- Convulsive movements
- Random movements of limbs / staggering / stationary and appearing confused

Try to attract their attention by knocking on the window. After assessing the risk to yourself open the door and gesture for them to leave. Never enter a room where the alarm is sounding and the level of Oxygen is below 19%.

## *Major spill of Liquid Nitrogen*

If a large amount of $LN_2$ has been released either due to the failure of a large pressurised dewar or a spill in a lift, the surrounding area should be evacuated.

Should the spill involve the release of more than 250 L it may be necessary to trigger the fire alarm to evacuate the area.

If the fire alarm is used to trigger an evacuation, the fire incident controller should be contacted on 0909 and informed of the nature of the emergency and informed where the spill has occurred and to stop members of the security and fire response team investigating the area.

The incident controller should be instructed to let the fire brigade know that $LN_2$ has been split and that breathing apparatus may be required.

## *Further Information*

Information on Cryopreservation is available from Thermo Fisher Scientific

**Safety Data Sheets for Cryogenic Gases**

SDS for different Liquefied gases are available from a variety of companies including Air Products, International Industrial Gases, and BOC Cryospeed. A SDS for Liquid Carbon Dioxide can be accessed from BOC. BOC also have a webpage on "Cryogenic Gas Risks" and a PDF on "Care with Cryogenics". Air products has a safety sheet on the "Safe handling of cryogenic liquids