Rankine Building

SAFE USE OF LIQUID NITROGEN (LN2)
Code of Practice (February 2017 update)

This Code of Practice is modified from the Cavendish Lab version

CONTENTS

INTRODUCTION

This document covers the transport, storage, use and disposal of liquid nitrogen. It sets the standards expected at the School of Engineering.

HAZARDS

There are two main types of hazard that liquid nitrogen (hereafter referred to as LN) presents: those related to temperature and those related to its conversion to nitrogen gas (vapour).

TEMPERATURE-RELATED HAZARDS

LN has a temperature of -196^{o}C. Severe cold burns can result from skin contact with the liquid or objects cooled by the liquid or its vapour. The skin will freeze to cold metal surfaces and may become detached on removal from the surface.

Many materials become brittle at this temperature and can shatter or crack.

Liquid oxygen may condense in containers of LN. **This raises the possibility of an explosive reaction with oxidisable material.**

VAPOUR-RELATED HAZARDS

LN expands around 700-fold when it vaporizes at room temperature (e.g. 1 litre of liquid produces nearly 700 litres of gas). Closed vessels containing LN may explode because of the build-up in pressure caused by the evaporation. In poorly-ventilated rooms there is a danger that air will be displaced by the nitrogen, leading to an oxygen-depleted atmosphere and death by asphyxiation.

Asphyxia – Effect of Oxygen Concentration	
OXYGEN (vol. %)	**EFFECTS AND SYMPTOMS**
18-21	No discernible effects can be detected by the individual.
11-18	Reduction of physical and intellectual performance without the sufferer being aware.
8-11	Possibility of fainting within a few minutes without warning.
6-8	Fainting occurs after a short time. Resuscitation possible if carried out immediately.
0-6	Fainting almost immediate. Brain damage may occur, even if resuscitated. Death.

The danger from very low oxygen levels (<6%) is that a person can become unconscious after two breaths – they do not even have time to realize that something is wrong. If they remain in that atmosphere for a few minutes, they die. Multiple fatalities are common in such circumstances because a passer-by or colleague, seeing someone collapsed, rushes into the room to render assistance but succeeds only in becoming another casualty. This type of event is not confined to a whole room. The areas covered do not have any low level duct areas where nitrogen levels could cause a problem

More information about the oxygen depletion, including data tables, are provided in Appendix A.

PRECAUTIONS
In addition to emergency actions, for example following splashes or spillages, which must be prepared for, there are five main activities that could lead to danger:

- Transport
- Storage
- Filling
- Use
- Disposal

These activities are covered in detail below.

RISK ASSESSMENT
The precautions that need to be taken will depend on individual circumstances and should be decided following a risk assessment (as required by both the Management of Health and Safety at Work Regulations 1999 and the Control of Substances Hazardous to Health Regulations 2002 (COSHH). As discussed below, some of the precautions are common to all users. **Appendix B** shows a risk assessment proforma that deals with the hazards and precautions for the use of LN in other areas away from levels 2 and 6; it does not cover other aspects of the work and individual risk assessments should be tailored to suit the particular circumstances. The form in **Appendix B** should be regarded as an addendum to the normal risk assessment form for individual labs. Appendix C contains standards for risk assessment.

TRANSPORT
In this document the term "transport" refers to movement of liquid nitrogen **within** the School of Engineering. Transport **to** the Campus is the responsibility of the supplier of liquid nitrogen, BOC.

Standard Operating Procedure (S.O.P) for Liquid Nitrogen vessels transport to the labs on levels 2 and 6

Liquid Nitrogen delivery to Rankine Building is arranged by either Bill Ward, Anne McGarrity or Julie Russell contacting BOC cryogenics delivery driver by phone and the 240 litre vessel(s) left outside in back lane. The vessel is transported using the motorised pallet truck. BOC cryogenics fill the vessel. The filled vessel is brought back into the building using the motorised truck. The back lane is on level 2 so no need for transportation of the dewar in the goods lift for level 2 dewar but the goods lift must be used for transport to level 6. A sign stating **'Liquid Nitrogen do not enter or touch'** is put on the vessel and the goods lift is called

- Call goods lift to level 2 using key switch.
- Load dewar/vessel into the goods lift, attach sign, and close lift doors
- Take passenger lift to level 6, call goods lift using keyswitch.
- Transfer dewar/vessel to level 6 storage area and fit coded padlock to valve, return motorised truck to level 2

NO-ONE SHOULD STAY IN LIFT WITH NITROGEN DEWAR DUE TO DANGER OF ASPHYXIATION

- Personal Protective Equipment (PPE) provided i.e. thermal gloves and face-shield must be worn when dispensing LN.

STORAGE

The danger is one of asphyxiation from nitrogen gas displacing the air. Storage outside the building would be ideal but no safe storage space is available. Storage inside the building is more of concern. The greatest danger is in the morning, because there could have been significant build-up of asphyxiant overnight and especially over the weekend. Although the oxygen deficiency is unlikely to be hazardous under normal conditions (see Table 1, Appendix A), there is still uncertainty about abnormal conditions, for instance the degradation of insulation leading to a larger-than-expected release of gas.

In the Rankine Building there are two 240 litre Dewars/vessels and these are located;
1) Level 6 in Room 604a (lift shaft) adjacent to external window with large air vents, local extraction to the roof and local Oxygen deficiency monitor and alarm.
2) Outside room 211 in corridor 213 adjacent to an extract fan with a capacity of 13 m^3 discharging outside.

The following are all **undesirable features** for a room in which LN is stored:

- poor ventilation;
- small size;
- large volume of LN;
- windowless door;
- no oxygen deficiency alarm.

See Appendix C for standards.

FILLING

VAPOUR-RELATED

Approximately 10% of the top-up liquid evaporates during the process. So, a 25 litre top-up produces 1707 litres of gas. From Table 2 in Appendix A it can be seen that filling has a much greater effect on oxygen levels than does normal storage. Table 2 shows the effect on

a room with 0.4 air changes an hour – this is a typical figure for a room without mechanical ventilation.
Authorised users will have the code for the coded padlock on the dewar, remove the padlock. Before doing anything further wear the leather elasticated gloves (PPE) provided. If using a polystyrene container place container on floor place diffuser tubing into the container and slowly open the valve to begin filling container, do not fully open the valve. Once almost filled close the valve and remove the feed tubing, replace the coded padlock and transport to the lab using stairwell if required.
If using 25 litre container, (onion) follow the above procedure, if it is necessary to move the container to another level this must be done using the goods lift and you should **not** travel in the lift with the container, use the key call system described above for moving 240 litre dewar.

TEMPERATURE-RELATED
Personal Protective Equipment (PPE) should be used with LN. See below for a description of PPE.

USE
In addition to concerns related to both temperature and vapour, there is a further **concern of infection.**

INFECTION
It has been reported in the scientific literature that LN tanks can become contaminated with viruses, bacteria, fungi and cells. Indeed, LN is generally the best method to preserve micro-organisms. In one incident, this contamination (Hepatitis B virus) was passed on to recipients of a bone marrow transplant. Although the samples in these reports were not stored in cryovials, the possibility must exist; if there is leakage of LN into a vial then it is reasonable to anticipate some transfer of material out of the vial into the surrounding liquid.
Hence the storage of cell lines from primary sources (which may be infected) in the same tank as cell lines from commercial sources should be avoided.
Storage of cryovials in the LN vapour phase is advised for good laboratory practice. Furthermore, the manufacturers of cryovials state that they should not be immersed in LN. This method also avoids contamination between samples.

VAPOUR-RELATED

Oxygen depletion
The main concern is from a spillage. The undesirable characteristics of a room are as for storage. Table 3 in Appendix A suggests that LN should not be used in rooms where the ratio of the room volume (m^3) to the LN volume (litre) is less than 15, unless there is good ventilation.

Explosion
Vessels containing LN should not be sealed because of the danger of explosion. Any caps/lids must be vented, with a sufficient aperture to prevent blocking by ice. Glass dewars should be taped so that in the event of an implosion, the glass fragments will be contained.
There have been incidents of exploding cryopreservation vials. If the vials are stored immersed in the liquid, it is possible for liquid to leak into the vial. When the vial is returned to room temperature, the LN inside it evaporates raising the pressure until the vial explodes. Vials with a female cap, a fine thread and no sealing ring have been known to explode. This type of vial should not be used. No incidents have been reported with another design in which the cap is male, has a thicker thread and has a sealing ring.

TEMPERATURE-RELATED
Personal Protective Equipment must be used but the parts of the body needing protection from splashes of LN will depend on how the LN is used.

DISPOSAL
Surplus LN should be allowed to evaporate naturally inside a fume cupboard or a well-ventilated area. LN must not be poured down the sink or drain.

TRAINING
Training is needed for everyone handling or using LN.

PERSONAL PROTECTIVE EQUIPMENT (PPE)
It should be remembered that PPE is not designed to withstand immersion in, nor prolonged contact with, cryogenic liquids.

Face
Splashing should be regarded as likely during any pouring operation. The PPE options are:
1. goggles
2. full-face visor
3. full-face visor with a chin guard.

Note:

- Eye protection should be to BS EN 166 grade 3.
- Safety spectacles do not give adequate protection against splashes and are NOT suitable.
- Goggles protect only the eyes. A visor protects the eyes and the face. However it is still possible for liquid to splash up underneath the visor. The use of a visor with a chin guard should be considered if this is likely to occur.

Hands should be protected by special gloves (to BS EN 511 (cold protection)).
The possibility of LN being **splashed into the glove via the cuff area** needs to be considered in the risk assessment. Loose, standard-length, gloves are more susceptible to splashing, especially if one hand is holding the receiving vessel while the other tips the storage dewar. (Ideally this eventuality should be designed out of the process – e.g. by providing a firm holder for the receiving vessel.) Alternatives are long-length gloves or standard gloves with elasticated cuffs.

Body
If splashing on to the body is likely, an apron should be worn. It should be from non-woven fabric and not have pockets. It should be long enough to protect the lower part of the legs.

Feet also need protection. Open-toed shoes and sandals are not suitable. If boots are worn, trousers should be worn outside the boots, to prevent liquid running into the top of the boot.

APPENDIX A

OXYGEN DEPLETION ARISING FROM EVAPORATION OF LIQUID NITROGEN

Liquid nitrogen evaporates slowly and could, in certain circumstances, displace enough of the air from the room to make the atmosphere dangerous to people.
There are three main sources of gaseous nitrogen:

- natural evaporation from the storage dewar
- evaporation during dewar filling and top-up
- spillage of LN

NATURAL EVAPORATION

Of particular concern are mornings because several hours will have elapsed since the room was entered, doors opened, etc.
The following calculation and Table 1 give an approximate idea of the degree of danger. The important parameters are:

- Volume of the room
- Volume of LN in store
- The number of air changes per hour
- The rate of LN evaporation, which depends on the integrity of the insulation

The standard equation for calculating nitrogen gas concentrations is:

$$C_N = L / V_R * n$$

Where C_N = increase in gas concentration after a period,
L = gas release (m^3/hr); V_R = room volume (m^3); n = air changes/hour
1 litre of LN produces 683 litres of gas.

The evaporation rate depends on the particular dewar. Manufacturers quote figures of around 0.8% per day for 25 litre vessels and 1.5% per day for 10 litre vessels. It is widely accepted that these figures should be doubled to allow for insulation degradation.

For example: at 0.8%/day, a 25 L dewar releases 11.4 litres of gas per hour. Table 1 shows the effect of normal evaporation on oxygen levels.

TOPPING-UP OR FILLING

Top-up or filling loses around 10% of the topping up volume so: 25 L top-up loses 2.5 L LN, producing 1707 litres of gas.

So filling dewars has much more effect on oxygen depletion than does normal storage. Table 2 shows the likely effects for a range of LN volumes and room sizes. The calculations assume an air change rate of 0.4 per hour – this is a typical figure for a room without mechanical ventilation.

SPILLAGE

Table 3 shows that spillage can have a dramatic effect on oxygen levels. For these calculations the effect of ventilation was ignored. Further assumptions are that the LN vaporises immediately and the released nitrogen gas mixes with the air. The figures therefore represent a pessimistic case.

Table 1: EVAPORATION DURING STORAGE
Effect of evaporation of LN on the oxygen concentration (%) in a room.

Room volume (m^3)	Volume of liquid nitrogen (litres)										
	10	25	50	75	100	150	200	250	300	400	500
15	21.0	21.0	20.9	20.9	20.8	20.8	20.7	20.6	20.5	20.4	20.2
25	21.0	21.0	21.0	20.9	20.9	20.9	20.8	20.8	20.7	20.6	20.5
50	21.0	21.0	21.0	21.0	21.0	20.9	20.9	20.9	20.9	20.8	20.8
75	21.0	21.0	21.0	21.0	21.0	20.9	20.9	20.9	20.9	20.9	20.9
100	21.0	21.0	21.0	21.0	21.0	21.0	21.0	20.9	20.9	20.9	20.9

The normal level of oxygen in the atmosphere is 21%. The above figures assume 0.4 air changes per hour. Shaded boxes represent low danger levels of oxygen depletion.

Table 2: FILLING
Effect of the filling of a LN dewar on the oxygen concentration (%) in a room.

Room volume (m^3)	Volume of liquid nitrogen (litres)										
	10	25	50	75	100	150	200	250	300	400	500
15	18.6	18.6	18.5	18.5	18.5	18.4	18.3	18.2	18.1	18.0	17.8
25	19.6	19.5	19.5	19.5	19.5	19.4	19.4	19.3	19.3	19.2	19.1
50	20.3	20.3	20.3	20.2	20.2	20.2	20.2	20.2	20.1	20.1	20.0
75	20.5	20.5	20.5	20.5	20.5	20.5	20.4	20.4	20.4	20.4	20.3
100	20.6	20.6	20.6	20.6	20.6	20.6	20.6	20.6	20.6	20.5	20.5

The normal level of oxygen in the atmosphere is 21%. The above figures assume 0.4 air changes per hour. Shaded boxes represent low and medium danger levels of oxygen depletion.

Table 3: SPILLAGE
Effect of the spillage of LN on the oxygen concentration (%) in a room.

Room volume (m^3)	Volume of liquid nitrogen spilt (litres)						
	1	2	3	4	5	10	25
15	19.6	18.1	16.7	15.3	13.8	6.7	
25	20.4	19.9	19.3	18.7	18.1	15.3	
50	20.7	20.4	20.1	19.9	19.6	18.1	
75	20.8	20.6	20.4	20.2	20.0	19.1	16.2
100	20.9	20.7	20.6	20.4	20.3	19.6	17.4

The normal level of oxygen in the atmosphere is 21%. The above figures assume 0.4 air changes per hour. Shaded boxes represent low, medium and high danger levels of oxygen depletion.

APPENDIX B

APPENDIX B – risk assessment proforma for liquid nitrogen [LN]
(This proforma is not a complete risk assessment for all work involving LN. It is only to be used in conjunction with full risk assessments for specific tasks/procedures/experiments and not as a substitute for them.)

Question	Yes/No/ NA	Information/ Comments
Storage		
Where is the LN stored (room number)?		
What is the volume of LN (litres) stored?		
Is there good natural ventilation in the room?		
Does the door of the room have a vision panel?		
Is the ratio of the room volume (m^3)/volume of LN (litre) in room >15?		
Is the air change rate in the room >0.5 changes per hour?		
Is there an oxygen depletion alarm in the room which is visible/audible from outside the room?		
Use		
Where is the LN used (room number)?		
What is the volume of LN (litres) used?		
Is there good natural ventilation in the room?		
Does the door of the room have a vision panel?		
Is the ratio of the room volume (m^3)/volume of LN (litre) in room >15?		
Is the air change rate in the room >0.5 changes per hour?		
Is there an oxygen depletion alarm in the room which is visible/audible from outside the room?		
PPE		
Is PPE provided to protect the following, where appropriate (give BS EN number)?:		
Face		
Hands		
Arms		
Body		
Feet		
Training		
Have all persons been given training as necessary in the transport, storage, use and disposal of LN?		
If you answered **No** to any of the above questions, you must stop using LN or change your arrangements for LN storage or use. If you answered **No** to training, contact Douglas Irons (ext 5251), Bill Ward (ext 6668) who can arrange on-line training courses in this area.		

APPENDIX C

APPENDIX C - STANDARDS

Risk assessment

1. A suitable and sufficient risk assessment shall be completed for all aspects of any work involving liquid nitrogen.

Ventilation in rooms

2. Wherever possible, LN should be stored in rooms having good natural ventilation.
3. The room must have an oxygen deficiency alarm installed inside the room.
   - the alarm signal must be audible/visible from outside the room.
   - the alarm must be checked periodically following the manufacturer's recommendations.

Training

4. Employees must be given adequate instruction and training on hazards, precautions, and emergency procedures associated with LN.

Personal protective equipment (PPE)

5. The personal protective equipment needed for the task must be identified in risk assessments.
6. The personal protective equipment identified in the risk assessment shall be:
   - provided and readily available;
   - suitable for each person having to use it;
   - be used properly at all relevant times;
   - be adequately maintained.

SITING OF LIQUID NITROGEN VESSELS WITHIN RANKINE BUILDING

ASSESSMENT OF RISK OF DANGEROUS LEVELS OF OXYGEN DEPLETION

To calculate oxygen concentration %:

$$\% O_2 = \frac{100 \times V_o}{V_r}$$

Where for nitrogen $V_o = 0.2095 (V_r - V_g)$

And for O_2 $V_o = 0.2095 (V_r - V_g) + V_g$

V_g = maximum gas release which is the liquid volume capacity of the vessel V x gas expansion

V_r = volume of the room

Gas expansion for N_2 = 682.7

<u>LOCATION 1- Level 2 Sub-corridor at level 2 (corridor 213)</u>

Assessment A - very worst scenario:

Room size is to nearest fire doors only
Room height is to suspended ceiling only
No allowance for mechanical ventilation
Full contents of vessels being released

Total volume of space: 128.25 cubic metres

Maximum volume of liquid nitrogen stored (1 full vessel) = 240litres

Calculation: V_r = 128.25 m3
V_g = 240 litres x 682.7 = 163848 litres = 163.85 cubic metres
V_o = 0.2095 (128.25 - 163.85) = -7.46
$\%O_2 = 100 \times \frac{(-7.46)}{128.25} = -5.8\%$ (Acceptable range 18 - 22%)

i.e No Oxygen can't have a minus % of gas

Assessment B - adjusted to account for:

Room size including level 2 corridor
Room height including void above suspended ceiling

Total volume of space: 391.95 cubic metres

Maximum volume of liquid nitrogen stored (1 full vessel) = 240litres

Calculation: V_r = 391.95 m3
V_g = 240 litres x 682.7 = 163848 litres = 163.85 cubic metres
V_o = 0.2095 (391.95 - 163.85) = 47.8
$\%O_2 = 100 \times \frac{47.8}{391.95} = 12.2\%$ (Acceptable range 18 - 22%)

This level of Oxygen is well below the threshold. See appendix A

Above calculations cover situations where the vessel releases all of its contents in a very short space of time. This is highly unlikely occurrence and would require excessive impact or malicious tampering or vandalism for this to happen

Due to the location of the vessel, and the close proximity of doors for the adjacent rooms, building a secure compound is not feasible. A coded padlock is attached to the valve handle preventing it being opened unless by an authorised user.

ASSESSMENT ASSUMING NORMAL ACTIVITY

In normal use the amount of gas released from a vessel will be determined by the integrity of insulation and the appropriate function of the controls and safety devices. All vessels in the Rankine Building are subject to periodic testing and inspection to ensure they operate within the manufacturer's specification. BOC Cryocare certify the vessels are fit for purpose

Assuming these vessels are within specification an acceptable loss of 2% liquid over a 24 hour period is considered the norm. This results in a vastly reduced gas volume released into the room. Taking the room volumes as those used in Assessment A the resultant effect would be

$Vr = 128.25\ m3$

$$Vg\ (\text{over 24 hour period}) = \frac{2 \times 240}{100}\ \text{litres} \times 682.7 = 3277\ \text{litres} = 3.3\ m3$$

$Vo = 0.2095\ (128.25 - 3.3) = 26.2$

$$\%O2 = \frac{100 \times 26.2}{128.25} = 20.42\%\ \ (\text{Acceptable range } 18 - 22\%)$$

This area has a window fan that moves 13m3 of air per min when running at full speed and is left running at all times. It would take 12.6 minutes to displace 164 cubic metres of Nitrogen.

In these circumstances the risk of low Oxygen level is minimal.

LOCATION 2- Level 6 ROOM 614a

Assessment A - very worst scenario:

Room is served by extract hood and vented window
Room height is 3.5 metres, width is 3.4 m, depth is 2.8m. Volume = $33m^3$
Mechanical ventilation
Full contents of vessels being released

Total volume of space: 33 cubic metres

Maximum volume of liquid nitrogen stored (1 full vessel) = 240litres

Calculation: $Vr = 33\ m^3$

$Vg = 240$ litres x 682.7 = 163848 litres = 163.85 cubic metres

$Vo = 0.2095\ (33 - 163.85) = -\ 130.85 \times 0.2095 = -27.4$

$$\%O2 = \frac{100 \times (-\ 27.4)}{33} = -83\%\ (\text{Acceptable range } 18 - 22\%)$$

i.e. No Oxygen in the room

Room 614a is at the end of a corridor with ventilation and external air supply. The door has warning sign telling everyone **NOT TO ENTER** if audible and/or visual alarm is activated.
The alarm will only go quiet once the Oxygen level goes back to normal.
Any Nitrogen gas escape into the stairwell will be diluted by the volume of air over 8 floors.

Above calculations cover situations where the vessel releases all of its contents in a very short period of time. This is a highly unlikely occurrence and would require excessive impact or wilful tampering and/or vandalism for this to happen.

CODE OF PRACTICE
TRANSPORT AND STORAGE OF CRYOGENIC LIQUIDS
IN THE RANKINE BUILDING

Cryogenic liquids are in use in various applications throughout the Rankine Building and portable storage vessels are located at the following designated areas:

Level	Location	Research Group	No. of Vessels	Capacity
6	Room 614a Lift shaft	Biomedical Engineering	1	240 litres
2	Sub corridor adjacent to room 211	Electronics & Nanoscale Engineering	1	240 litres

[*N.B. 1 liquid litre of Nitrogen will expand to 696 litres of gas at room temperature*]

The above areas have forced ventilation; level 2 has a window fan running continuously moving air at a rate of 13 cubic metres per minute and level 6 has ventilation linked with the extract hood in the room as well as vents in window frame.

Risk assessments show that boil off rates in storage do not present any risk of oxygen depletion at these locations but a full vessel discharge could present a low oxygen hazard. This is regarded as highly unlikely but both locations have low oxygen alarm systems in place as a precaution.

The liquid nitrogen vessels are transported from their location to the fill point, Rankine rear lane, using the motorised pallet truck and goods lift as required. Liquid Helium is rarely used but when required this is delivered in a rented vessel normally supplied by BOC. Deliveries of liquid nitrogen are pre-arranged with the BOC Cryospeed driver and the driver is responsible for the filling process. Vessels are taken out before the delivery driver arrives and returned to their storage location after filling.

The following protocol must be adhered to when transporting vessels:

- Every effort should be made to avoid times when lifts and corridors are busy e.g. student classes, visitors, lunch breaks.
- The electric pallet truck must be used to transport vessels. Training in the correct use of this equipment can be arranged through Bill Ward ext. 6668
- Transport of polystyrene buckets containing liquid nitrogen should be by the south stairwell - the lift must not be used.
- People should NEVER travel in the lift with a vessel containing cryogenic liquids.
- The goods lift key should be used to call the lift to load the vessel and also to call the lift to the destination level (Lift control keys are held by the user group).
- A warning notice stating;
  'CRYOGENIC LIQUID IN TRANSIT - DO NOT ENTER AND DO NOT TOUCH'
  MUST be attached to vessels containing liquid being transported in the goods lift.
- The transportation of cryogenic liquids can only be carried out by people who have appropriate knowledge, training and experience in this area. These people, who will be deemed to be competent, will be aware of the hazards involved and how to cope with them.
- Appropriate Personal Protective Equipment must be worn i.e. cryogenic gloves, safety footwear and lab coat.

For further guidance on handling cryogenic liquids in the School please contact either Bill Ward ext 6668, one of the Technical Managers or Andrew Glidle, School Safety Director.

Updated on 9th February 2017