Victoria University of Wellington
School of Biological Sciences

Safe Method of Use - LIQUID NITROGEN

CONTENTS

INTRODUCTION

This document covers the transport, storage, use and disposal of liquid nitrogen. It sets the standards expected within SBS*. This should be used to when developing a safe method of use for specific methods/activities.*

HAZARDS

There are two broad types of hazard arising from liquid nitrogen (LN) –those related to temperature and those related to vapour.

1. 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 vapour.

   The skin will freeze to cold metal surfaces.

   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.

2. VAPOUR-RELATED HAZARDS

   LN expands around 700-fold when it vaporises at room temperature (eg 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-deficient atmosphere and death by asphyxiation.

Asphyxia – Effect of O_2 Concentration	
O_2 (vol %)	**Effects and Symptoms**
18-21	No discernible symptoms 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 prior warning. **Risk of death below 11 vol%.**
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 rescued.

The danger from very low oxygen levels (ca. 6% or less) is that you become unconscious after two breaths – you do not even have time to realise that something is wrong. If you remain in that atmosphere for a few minutes you 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 a casualty.

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:

1. Transport
2. Storage
3. Filling
4. Use
5. Disposal

They are discussed in detail below.

The precautions that need to be taken will depend to some extent on individual circumstances and should be decided following a risk assessment (as required by both the Health and Safety at Work Act 2015 and the Health and Safety at Work Hazardous Substances Regulations 2017). As discussed below, some of the precautions are common to all users.

Appendix B shows some factors to consider when developing a risk assessment. These deal with the general hazards and precautions for the use of LN; it does not cover other aspects of the work and individual risk assessments should be tailored to suit the particular circumstances. The factors in Appendix B should be regarded as a starting point to the normal experiment/project based risk assessment.

Appendix C contains standards for risk assessment.

1. TRANSPORT
   - INSIDE THE BUILDING

     The transport of LN via the stairs is potentially dangerous. A spillage of LN, for example from the container being dropped or overturned, could result in cryogenic liquid flowing down many flights of stairs.
     If LN is transported in a lift with solid doors, the lift becomes a confined space. The danger is that the lift will breakdown between floors and that the LN will boil off, displacing the oxygen in the lift, and causing the death of the people in the lift.
     Hence it is not advisable for people to travel in the lift with the LN dewar. The lift at the South end of the TTR building has the potential to be controlled remotely, thus allowing dangerous goods to be transported without being accompanied.

   - ROAD TRANSPORTATION OF SAMPLES IN LN

     This is a potentially hazardous activity because of evaporation of nitrogen into the vehicle. The LN container should not be in the same compartment as the driver and passengers, which means that hatchbacks, minibuses and most vans are not suitable. To avoid the effects of leakage of nitrogen gas from the storage compartment into the passenger compartment, the driver should keep a steady flow of air into the car. The standards in Appendix C apply.

2. STORAGE

The danger is one of asphyxiation from nitrogen gas displacing the air. With storage inside the building the main concern 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, there is still uncertainty about abnormal conditions, for instance the degradation of insulation leading to a larger-than-expected release of gas.

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.

3. FILLING

- VAPOUR-RELATED

  Approximately 10% of the top-up liquid evaporates during the process. So, a 25litre top-up produces 1707 litres of gas. From tables 2a and 2b in Appendix A it can be seen that filling has a much greater effect on oxygen levels than does normal storage. Table 2a shows the effect on a room with 0.4 air changes an hour – this is a typical figure for a room without mechanical ventilation. Table 2b shows the beneficial effect of increasing the air flow rate to 1 air change an hour.

- TEMPERATURE-RELATED

  Personal Protective Equipment (PPE) should be the last resort, but will almost always be needed when LN is used. See below for a description of PPE.

4. USE

Staff should not handle liquid nitrogen in any confined space, when alone. All out of hours use of liquid nitrogen should be avoided if possible and, in any case, should be fully risk assessed in advance.

- INFECTION

  It has been reported in the scientific literature that LN tanks can become contaminated with viruses, bacteria, fungi and cells. 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 <u>into</u> a vial then it is reasonable to anticipate some transfer of material <u>out of</u> 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.

Tedder, R.S., Zuckerman, M.A., Brink, N.S., Goldstone, A. H., Fielding, A., Blair, S., Patterson, K.G., Hawkins, A. E., Gormon, A.M., Heptonstall, J., Irwin, D. 1995. Hepatitis B transmission from contaminated cryopreservation tank. Lancet 346:8968, 137-140.

Grout, B. W. W. and Morris, G. J. 2009. Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer. Theriogenology 71:1079-1082.

- 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 (m3) to the LN volume (litre) is less than 15 unless there is a 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.
    If cryopreservation 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.

- TEMPERATURE-RELATED
  Personal Protective Equipment should be the last resort, but will almost always be needed when LN is used. The parts of the body needing protection from splashes of LN will depend on how the LN is used.

5. DISPOSAL
   Surplus LN should be allowed to evaporate naturally inside a fume hood or a well-ventilated area. If leaving surplus liquid nitrogen to evaporate display a caution notice informing other lab users of the hazardous contents.
   LN must not be poured down the sink or drain.

6. TRAINING
   Training is needed for everyone handling / using LN.

7. PERSONAL PROTECTIVE EQUIPMENT
   It should be remembered that PPE is not designed to withstand immersion in, or prolonged contact with, cryogenic liquids.

   Face
   Splashing should be regarded as likely during any pouring operation. The PPE options are:
   - Safety glasses
   - Goggles
   - Full-face visor
   - Full-face visor with a chin guard.

   Note:
   Safety glasses may not give adequate protection against splashes and should not be used during any decanting of LN
   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.

Hands

Hands should be protected by special cryoprotective gloves. 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 – eg by providing a firm holder for the receiving vessel.) Alternatives are long-length gloves or standard gloves with elasticated cuffs.

When NOT handling LN directly but rather when handling items that have previously been exposed to LN e.g. cryotubes, and where high level of dexterity is required then the use of disposable gloves is permitted

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

Feet also need protection. Closed shoes suitable for normal laboratory use should be used. If boots are worn, trousers should be worn outside the boots, to prevent liquid running into the top of the boot.

See Appendix C for the standard on PPE.

Emergency response

1. CRYOGENIC BURNS

   Cryogenic burns are cold burns caused by liquefied gases such as LN

   Burns caused by contact with LN may not be immediately apparent but can develop some time later. The skin will appear white and waxy. The affected area should be thawed slowly. Place affected area under cool running water, **never hot**, for at least 10 minutes then cover with a sterile dressing. If any clothing etc. is frozen onto the skin, do not attempt to remove it until the area has been completely thawed. If the burn is extensive or deep (indicated by a loss of sensation in the area) then seek medical advice

2. SPILLAGE

   Be aware of, and ready to implement, emergency procedures for spills.
   In the event of a spill any liquid will rapidly turn to nitrogen gas due to vaporisation. If working in a well-ventilated area small volumes should not present an asphyxiation hazard. In the event of a larger spill concentrations of oxygen in the air will fall. Evacuate the area and prevent access for a few minutes to allow the atmosphere to return to normal. Do not close the door. Open windows if safe to do so.

3. EQUIPMENT

   If equipment (dewars, thermos flasks, dry shippers, dewar trolley, any PPE) does not appear to be functioning correctly, report immediately.

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

1. 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:
CN = L / VR * n
Where CN = increase in gas concentration after a long period; L = gas release (m3/hr); VR = room volume (m3); 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.

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

2. TOPPING-UP / FILLING

Top-up / filling loses around 10% of the topping up volume so: 25l top-up loses 2.5l LN, producing 1707 litres of gas.
So filling dewars has much more effect on oxygen depletion than does normal storage. Table 2a 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. Table 2b shows the effect of increasing the ventilation to 1 air change an hour.

3. 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.

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**

Table 1: oxygen concentration, %: effect of evaporation; 0.4 air changes/hour

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**	**18.2**	**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**	**20.2**	**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**

Table 2a: oxygen concentration, %:
effect of topping-up with 10litres LN + evaporation; 0.4 air changes/hour

Room Volume (m^3)	Volume of liquid nitrogen, litres										
	10	25	50	75	100	150	200	250	300	400	500
15	**20.0**	**20.0**	**20.0**	**20.0**	**20.0**	**19.9**	**19.9**	**19.9**	**19.9**	**19.8**	**19.7**
25	**20.4**	**20.4**	**20.4**	**20.4**	**20.4**	**20.4**	**20.3**	**20.3**	**20.3**	**20.3**	**20.2**
50	**20.7**	**20.7**	**20.7**	**20.7**	**20.7**	**20.7**	**20.7**	**20.7**	**20.7**	**20.6**	**20.6**
75	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**
100	**20.9**	**20.9**	**20.9**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**	**20.8**

Table 2b: oxygen concentration, %:
effect of topping-up with 10litres LN + evaporation; 1 air change/hour

Room Volume (m^3)	Volume of liquid nitrogen spilled, 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**

Table 3: oxygen concentration, %: effect of spillage

APPENDIX B

FACTORS TO CONSIDER WHEN DETERMINING RISK:

1. **Storage**

   Location of store (with room number)
   What is the volume of LN (litre)?
   What is the volume of room (m^3)?
   Is there good natural ventilation?
   Is the air change rate in room >0.5 per hour?
   Is there an oxygen depletion alarm?
   If yes:
   · Is it audible/visible from outside?
   · Is a malfunction audible/visible from outside?
   · Is it checked periodically according to manufacturer's recommendations?

2. **Transport**

   How do you transport LN within the building?
   How do you transport LN to and from field sites?
   Which vehicle do you use?

3. **Use**

   Location (eg lab name/room number, floor)
   Is there good natural ventilation?
   Is the ratio of room volume (m3)/volume LN (litre) in room >15?
   Is the air change rate in room >0.5 per hour?
   Is there an oxygen depletion alarm?
   If yes:
   · is it audible/visible from outside?
   · is a malfunction audible/visible from outside?
   · is it checked periodically according to manufacturer's recommendations?

4. **PPE**

   What PPE is provided to protect the following: Face, Hands, Arms, Body & Feet?
   Have users received documented training in the correct use and maintenance of PPE?

5. **Training**

   Have all users received documented training (as necessary) in the transport, use and disposal of LN?

APPENDIX C - STANDARDS

risk assessment

1. A suitable and sufficient risk assessment shall be completed for all aspects of the use of liquid nitrogen.

ventilation in rooms

2. Wherever possible, LN should be stored in rooms having good natural ventilation.
3. Where this is not possible:
   - the room should have forced ventilation of at least 1 air change an hour.
   - the room should have an oxygen deficiency alarm installed inside the room.
   - the alarm signal should be audible/visible from outside the room.
   - alarm malfunctions should be visible/audible from outside the room.
   - the alarm should be checked periodically following the manufacturer's recommendations.

transport in road vehicles

4. The liquid nitrogen container should be in a separate compartment from the driver and passengers.
5. The container must be secured to prevent movement during travel.
6. The container must be labelled adequately
7. There must be a constant airflow into the passenger compartment of the vehicle.
8. The driver must be made aware of the hazards associated with liquid nitrogen.
9. The driver must be given written information containing details of:
   - Contact names and numbers at the hospital
   - What to do in the event of a spillage
   - What to do in the event of an incident
   - What to do in the event of contamination to passengers or themselves.

training

10. Users must be given adequate instruction and training (which is documented) on the hazards, precautions, and the actions required under the laboratory's emergency response plan in the event of an accident or accidental exposure to the substance.

personal protective equipment

11. The personal protective equipment needed for the task shall be identified in the risk assessment
12. The personal protective equipment identified in the risk assessment shall be
    - provided.
    - suitable for each person having to use it i.e. one size DOES NOT fit all.
    - be used properly at all relevant times.
    - be adequately maintained.