RISK ASSESSMENT:
HANDLING, TRANSPORTATION AND STORAGE OF LIQUID NITROGEN AND OTHER CRYOGENIC MATERIAL

More information can be found in the liquid nitrogen section of the departmental Health & Safety webpages: http://www.york.ac.uk/biology/intranet/health-safety/liquid-nitrogen/

Author of assessment:
Date of assessment:
Review Date:

Summary of laboratory activities involving use of liquid nitrogen:
- The laboratory are using liquid nitrogen to store cells long term and to snap freeze tissues
- The laboratory has two large storage Dewars and a 50litre pressurised vessel for topping
- In the equipment room there are two Dewars, one is a large storage Dewar and a 25litre non-pressurised Dewar for experimental use in the lab.

| Risk Assessment Review: Required at least once a year or immediately following any significant change to procedure |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Date Due | Date Conducted | Date Due | Date Conducted |
| Review 1 | Review 2 | Review 3 | Review 4 |

Properties of liquid nitrogen (liquid N2)

- A colourless, odourless liquid
- Extremely cold
- Boiling point is -196°C
- Nitrogen gas is evolved which is neither toxic nor harmful
- Small volumes vaporize to give large volumes of gas (1litre gives 0.7 m$^3$ of gas) which will displace oxygen in air.

Known or Expected Hazards

a) Temperature Related

- The extremely low temperature of liquid nitrogen can cause severe burn-like damage to the skin either by contact with the fluid, surfaces cooled by the fluid or evolving gases. The hazard level is comparable to that of handling boiling water.
- The low temperature of the vapour can cause damage to softer tissues e.g. eyes and lungs but may not affect the skin during short exposure.
- Skin can freeze and adhere to liquid nitrogen cooled surfaces causing tearing on removal.
- Soft materials e.g. rubber and plastics become brittle when cooled by liquid nitrogen and may shatter unexpectedly.
- **Liquid oxygen** may condense in containers of liquid nitrogen or vessels cooled by liquid nitrogen. This can be extremely hazardous because of the pressure rise on the slightest degree of warming above the boiling point of oxygen (-183°C) and the possibility of **explosive reaction** with oxidisable material.
- Thermal stress damage can be caused to containers because of large, rapid changes of temperature.

b) Vapour Related

- Large volumes of nitrogen gas are evolved from small volumes of liquid nitrogen (1 litre of liquid giving 0.7 m³ of vapour) and this can easily replace normal air in poorly ventilated areas leading to the danger of asphyxiation. It should be noted that oxygen normally constitutes 21% of air.
- Atmospheres containing less than 10% oxygen can result in brain damage and death (the gasping reflex is triggered by excess carbon dioxide and not by shortage of oxygen), levels of 18% or less are dangerous and entry into regions with levels less than 20% is not recommended. (Notices are posted in the stairwell altering lab workers to the dangers in case of Oxygen deficiency alarm sounding)
- Oxygen condensed into leaking containers can explode on heating following resealing or blockage with ice.

**Risk Evaluation and Control Measures**

For an untrained person, the risk of injury is moderate with cryogenic burns the most likely injury. However in exceptional circumstances when large amounts of material are spilled in an enclosed space, asphyxiation could occur which may be fatal.

**Particular Group of Workers Who May be at Increased Risk**

The most likely injury is to the person handling liquid nitrogen although following major spillage all inhabitants in the vicinity may be affected. Spillage may occur when filling the storage Dewars in XXX, there is an oxygen level monitor (linked to a large fan situated at ground level) to prevent anyone entering the room if the oxygen levels are low.

**First Aid**

Information can be found in the liquid nitrogen section of the departmental Health & Safety webpages: [http://www.york.ac.uk/biology/intranet/health-safety/liquid-nitrogen/first-aid/](http://www.york.ac.uk/biology/intranet/health-safety/liquid-nitrogen/first-aid/)

**Personal Protective Equipment**

Information can be found in the liquid nitrogen section of the departmental Health & Safety webpages: [http://www.york.ac.uk/biology/intranet/health-safety/liquid-nitrogen/ppe-ln2/](http://www.york.ac.uk/biology/intranet/health-safety/liquid-nitrogen/ppe-ln2/)

**Face protection, gloves and a laboratory coat must be worn when dispensing liquid nitrogen.**
Two full face visors and two pairs of insulated gloves are located next to the Dewars.
Emergency Procedures

Area 1
- In the event of a spillage or accidental release of a significant quantity of liquid nitrogen, more than 5 litres, the oxygen level will decrease causing the monitor to be activated, sounding an alarm, warning personnel not to enter the room.
- The alarm will also switch on the fan which is situated at ground level, to expel air to the outside of the building.
- Also follow the procedure detailed below.

Area 2
In the event of a spillage or accidental release of a significant quantity of liquid nitrogen, more than 5 litres, follow the procedure detailed below.

- Evacuate the area. Deploy warning signs at each end of the corridor.
- The area will be ventilating as fans are permanently running for the -80º freezers.
- Try to stop the release if at all possible e.g. turn off valves, but only if it is safe to do so - always wear protective clothing.
- Do not re-enter area unless it is safe to do so. Wait until all the liquid nitrogen has evaporated and at least 2 air changes have occurred. This would be approximately 30 minutes, as it is a large open space with good ventilation.
- Prevent liquid nitrogen from entering drains, basements, pits or any confined space where accumulation may be dangerous.

Removal and Storage of cryovials in liquid nitrogen
This involves removing racks and boxes immersed in liquid N₂.
When removing racks care should be taken to ensure that eyes are protected at all times: a face shield or safety glasses, dry insulated gloves that are easily removable and lab coat should always be worn. Never immerse your fingers in liquid nitrogen even for a second.
When holding containers with liquid nitrogen or anything that has been in contact with liquid nitrogen, make sure that you work fast and keep exposure to a minimum.
Do not use brittle plastics or glass vials which may shatter on contact with the cold liquid.

Disposal of liquid nitrogen
- Liquid nitrogen should be disposed of by evaporation.
- Do not pour it down the sink ever.
- The container should be left either in a fume hood with the door closed or in a well ventilated large laboratory, with the lid off.
- In a laboratory leave it at the back of a bench or in a fume hood, propped up or in a container if necessary to prevent it being knocked over.
- Do not leave it on the floor.
- Label it clearly so the cleaners know not to touch it/knock it over.

Transportation of liquid nitrogen
Anyone transporting Dewars should wear personal protective clothing, including lab coats, face mask and insulated gloves (see section above).

Maintenance
All pressurised vessels are inspected periodically by a trained engineer appointed by the insurance company responsible and all records of inspections filed. Any obvious damage is to be reported to
Laboratory Manager, a note should be attached to the vessel that it is out of use until an inspection has been carried out.

An oxygen deficiency monitor and forced ventilation system are fitted, supplied and serviced by XX. Service carried out twice per year, service records kept by XXX.

A quick test of the oxygen monitor should be routine whilst filling up the storage Dewars. This can be done by pressing the test button and observing any lights being activated, the display should stay blank.

Training

Main Facility
The Department is responsible to provide training the users of the main storage facility and all users of this facility must receive training before use. Training will include awareness of the properties and hazards of liquid nitrogen in addition to local departmental procedures for usage, storage and transportation.

All users of the departmental storage Dewars in room B/E/012 must be authorised. Please contact biol-infrastructure-group@york.ac.uk to arrange training before use.

A supplementary ‘practical training workshop’ is also organised by the University; details of available courses can be found at http://www.york.ac.uk/admin/pod/section.cfm?section_id=40.

Laboratory
The CII Laboratory Manager is responsible for the training of users of the CII storage Dewars and 50L pressurised vessel.

In addition to the departmental procedures, the laboratory has a procedure for filling liquid nitrogen Dewars, this procedure is attached (Appendix II).

There are also Key Working Practices displayed in XXX

All new CII staff will be trained by XXXX before handling liquid nitrogen.

All liquid nitrogen users should read this risk assessment together with the departmental local rules and sign the form in the file, to indicate that they have read the local rules.

Assessment of Ventilation Requirements

Area 1
Normal Working Practice

Area 1 (H=2.8m, W=2.6m, D=4.5) these measurements only take the ground floor space into account. H028 houses 2x large storage Dewars, non-pressurised liquid nitrogen vessels and 1x pressurised storage vessel, capacity 50L. The rate of evaporation from the vessels is 0.63L / 24 hours for large Dewars and 0.25L / 24 hour for pressurised container. The figure is also multiplied by 2 to allow for deterioration in the vacuum insulation with time. The room is above ground but has no windows and is estimated to have 0.5 air changes per hour by natural ventilation (there are two doors, one lending to the outside and the other to a large corridor).
L = (2 x 0.63 + 1 x 0.25) x 2 x 683
24 x 1000 = 0.086 m³ / h

V = 2.8 x 2.6 x 4.7 = 34.22 m³

N = 0.5

Therefore:
C = \frac{0.086 x 100}{34.22} = 0.2513\%

The nitrogen concentration in Area 1 is increased by 0.26%. The normal oxygen content of the atmosphere is approximately 21%, therefore:

\frac{21 x 100}{100 + 0.2513} = 0.9975\%

Under these circumstances the evaporation from the vessels in negligible and well within the safe working limits.

Oxygen Deficiency after a Large Spillage

Using the largest storage vessel as the worst case scenario, of 50L (IIU storage vessels are never filled above a certain line, samples kept under gaseous phase)

Room volume (Vr) = 2.8 x 2.6 x 4.7 = 34.22 m³

Maximum Gas release (Vg) = 50 x 683 = 34,150 litres = 34.15 m³

Vo = 0.2095 (34.22 – 34.15) = 0.0147

%O₂ = \frac{100 x .0147}{34.22} = 0.04%

The oxygen content of the room is reduced to 0.04%

As the calculation suggests the oxygen will be depleted to less than 18%, therefore an oxygen depletion monitor is permanently active and triggers a fan to expel air outside. There is also a fire door that can be opened to aide ventilation

Area 2

Normal Working Practice.

Area 2 (H=2.2m, W=4.0m, D=5.0m) which is part of the CAT2 corridor as there are no doors, corridor dimensions H=2.2m, W=2.0m, D=20m, these measurements only take the ground floor space into account. H015 houses 1x large storage Dewar, non-pressurised liquid nitrogen vessels and 1x non pressurised storage vessel, capacity 25L. The rate of evaporation from the vessels is 0.63L / 24 hours for the large Dewar and 0.25L / 24 hour for non-pressurised 25L container. The figure is also multiplied by 2 to allow for deterioration in the vacuum insulation with time. The room is above ground, has no windows, but has 2 large fans running permanently to keep the room cool for the -80°C freezers and is estimated to have 0.5 air changes per hour by natural ventilation (there are no doors in the room, it adjoins a large corridor with a door at either end.

L = (1 x 0.63 + 1 x 0.25) x 2 x 683
24 x 1000 = 0.050 m³ / h
\[
24 \times 1000
\]
\[
V = 2.2 \times 4.0 \times 5.0 + 2.2 \times 2.0 \times 20 = 132 \text{ m}^3
\]

\[N = 0.5\]

Therefore:
\[
C = \frac{0.050 \times 100}{132.00} = 0.038\%
\]

The nitrogen concentration in H015 is increased by 0.446%. The normal oxygen content of the atmosphere is approximately 21%, therefore:
\[
21 \times \frac{100}{100 + 0.38} = 20.99\%
\]

Under these circumstances the evaporation from the vessels in negligible and well within the safe working limits.

**Oxygen Deficiency after a Large Spillage**

Using the largest storage vessel as the worst case scenario, of 50L (storage vessels are never filled above a certain line, samples kept under gaseous phase)

\[
\begin{align*}
\text{Room volume (Vr)} &\quad 2.2 \times 4.0 \times 5.0 + 2.2 \times 2.0 \times 20 = 132 \text{ m}^3 \\
\text{Maximum Gas release (Vg)} &\quad 50 \times 683 = 34,150 \text{ litres} = 34.15 \text{ m}^3 \\
\text{Vo} &\quad 0.2095 \times (132 - 34.15) = 20.50 \\
\%O_2 &\quad 100 \times \frac{20.50}{132} = 15.5\%
\end{align*}
\]

The oxygen content of the room is reduced to 15.5%

As the calculation suggests the oxygen will be depleted to less than 18%, therefore ventilation fans are left running constantly. There is also a fire door that can be opened to aide ventilation at the end of the corridor.
Appendix I

Filling Dewars

Please also refer to Key Working Practice, displayed in XXX

- It is recommended that you are accompanied when filling liquid nitrogen Dewars.
- When filling from a pressurised storage Dewar, point the hose away from your face and body.
- Warm Dewars should be filled slowly to reduce temperature shock effects and to minimise splashing.
- Use a hose with a “phase separator” on the end which reduces splashing.
- Open valves slowly and prevent the valve from freezing up by moving the handle as the vessel is filling.
- Do not leave vessels unattended when filling.
- Ensure there is someone else around when filling containers, in case of accident.
- When filling from a pouring storage Dewar ensure that you are in a well-ventilated area of the lab and wearing all the necessary personal protective equipment.

A quick test of the oxygen monitor should be routine whilst filling up the storage Dewars. This can be done by pressing the test button and observing any lights being activated, the display should stay blank.
Appendix II

Liquid Nitrogen Storage Dewars

Liquid Nitrogen Oxygen Level Alarm System
The storage area for the Liquid Nitrogen Dewars has an oxygen monitor installed, due to the danger of asphyxiation from an LN₂ leak. This system samples the O₂ content of the air and if drops below a set level (around 20) this sounds an alarm and switches on a fan to extract the LN₂ vapour and vent it outside the building. This system does not automatically reset, so if the alarm goes off the back stairwell must be evacuated and closed off, and left for at least 15 minutes. After that time or when no LN₂ vapour is visible; if the O₂ level has risen back up to 20, reset the alarm by pressing the reset button, if not get out and try again in another 15 minutes.

Testing the fan
Once a week the fan on the LN₂ alarm system requires testing to ensure it doesn’t seize up. To do this press the button labelled ‘Press to test fan’ above the alarm system. Record the test on the sheet below the alarm system, and report any faults to XX.

Topping Up the Liquid Nitrogen Dewars in CII
You must wear a lab coat, face mask and cryogenic gloves when filling the tanks.

Usually the tanks are topped up every two weeks.

If you hear an audible alarm from any of the tanks please fill up the tank immediately, if you have been trained to use the equipment, or inform XXX

To check the level of the liquid nitrogen, dip the black measuring stick into the tank for a few seconds. Lift the stick out and give it a quick swipe backwards and forwards. You will then see a line on the stick. The tanks should be full to at least the red numbers (11” / 28cm).

If the level is low, use the pressurised container to manually fill. Make sure the hose is placed well into the tank and turn the liquid lever up 90°.

Fill until the level is up to the red numbers on the stick; try not to over fill the tank.

Cells are stored in vapour phase LN₂, with the bottom 3rd of the tank containing liquid nitrogen.

Topping Up the Liquid Nitrogen Pressurised Dewar
The pressurised Dewar is filled up on a Tuesday; please wheel it down to the back of Biology stores by 9am. Make sure the label is displayed with the account code.

The BOC tanker usually has all the Dewars filled by lunchtime, so you must remember to go and collect the Dewar on Tuesday, otherwise it will be left outside overnight and will not be secure.

Leave the pressurised Dewar for at least 2 hours before you use it, to let the pressure build up and equalise in the tank.

This vessel should never be used for filling small Dewars, due to the high pressure of the LN₂

Non-Pressurised Liquid Nitrogen Vessel
This is the blue vessel stored next to the freezers in the Main Lab; it is filled as required.
This vessel is used to fill small Dewars for research purposes.

The key code to open the store is XXX.

The large Dewar tank with the hose on is the one to use, place the hose well into the Dewar. Turn the knob but not completely, leave a bit of slack, whilst the Dewar is filling turn the knob occasionally and very slightly so it doesn't freeze up.

Once the tank is full (it holds 25 litres), write down the grant code, amount, name and date on the clipboard.

Instruction on the filling of the liquid nitrogen Dewars given by:-

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I have read & understood this document:-

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