Imperial College
London

X-ray Crystallography Facility, Room 5.20, Flowers Building
Working with liquid nitrogen

Local Rules

Created by Dr Liz Carpenter (31 March, 2003)
Revised by Dr Andreas Förster (9 Jul 2013)

1 Properties of liquid nitrogen (liquid N_2)

- A colourless, odourless liquid.
- Extremely cold.
- Boiling point is -196^{o}C.
- Upon vaporization, the volume expands 700-fold, displacing oxygen in the air.
- Nitrogen gas is the major component of air and is neither toxic nor harmful.

2 Hazards: Extreme low temperature and asphyxiation

2.1 Extreme low temperature hazards

Cryogenic burns

Liquid nitrogen can cause cryogenic burns if the substance itself, or surfaces which are or have been in contact with it (e.g. metal transfer hoses), come into contact with the skin. Local pain may be felt as the skin cools, though intense pain can occur when cold burns thaw and, if the area affected is large enough, the person may go into shock.

Frostbite

Continued exposure of unprotected flesh to cold atmospheres can result in frostbite. There is usually sufficient warning by local pain whilst the freezing action is taking place.

Hypothermia

Low air temperatures arising from the proximity of liquefied gases can cause hypothermia. Susceptibility is dependent upon temperature, exposure time and the individual concerned (older people are more likely to succumb).

2.2 Risk of asphyxiation

Nitrogen gas can displace O_2 in the air. Normally air contains 78% N_2, 21 % O_2 and 1 % argon gas. An atmosphere containing less than 18 % O_2 is potentially hazardous and should be avoided.

O_2 concentration in air	**Consequences**
21 %	Normal
11 – 14 %	physical and intellectual performance impairment, without the person being aware
8 – 14 %	possibility of fainting without prior warning.

6 – 8 %	fainting within a few minutes – resuscitation possible if carried out quickly.
0 – 6 %	fainting almost immediate, death ensues, brain damage if resuscitated.

How much nitrogen would you have to spill to get to these levels in various rooms?

O_2 concentration	**Consequences**	**Flowers good lift**	**3 x 5 meter lab (eg a cold room)**	**X-ray lab 5.20 Flowers**
18 %	Recommended minimum	2 litres	6 litres	36 litres
11 – 14 %	Confusion	5 litres	16 litres	60 litres
8 – 11 %	Possible fainting	6.5 litres	23 litres	125 litres
6 – 8 %	Immediate fainting	8.5 litres	30 litres	
0 – 6 %	Death	10 litres	35 litres	

E.g. One tall thin silver open dewar holds 3 litres of liquid nitrogen

Dry dewars full of liquid N_2 hold 10 litres

Storage dewars hold around 30 litres

Do not transport liquid N_2 in the Flowers Building goods lift when people are in the lift. Spilling the contents of a 10 litre Dewar would kill or cause brain damage for anyone in the lift. Instead, use the safety barrier to prevent access to the lift when transporting liquid nitrogen and take the stairs or the other lift to recover the Dewar.

In small labs/cold rooms a large, 35 litres storage dewar loosing vacuum and releasing its contents would be lethal. A couple of 3 litre dewars for freezing crystals would not be a problem.

The chances of asphyxiation in room 5.20 are minimal. The largest pressurized storage dewars hold 120 litres. If the whole contents discharged it would not cause damage to people in the room.

3 Safety measures

3.1 Low oxygen alarm

Room 5.20 is equipped with an oxygen monitor that will sound a piercing alarm if oxygen drops below 20%. While this is by no means dangerously low, evacuate the room immediately. If the alarm is due to low oxygen (instead of instrument failure), the alarm will silence after a few minutes when the air conditioning has restored oxygen to a normal level. Report all alarms to the facility manager.

3.2 Personal protective equipment

Always wear personal protective equipment as described below. In particular always protect your eyes.

- **Hands** - non-absorbent insulated cryogloves must always be worn when handling anything that is or has been in recent contact with liquid nitrogen. Cryogenic gloves are designed to be used in the vapour phase only and should not be

immersed into liquid nitrogen under any circumstances. They should be a loose fit to facilitate easy removal. Gauntlet style gloves are not recommended as liquid can drip into them and become trapped against the skin - sleeves should cover the ends of gloves or alternatively, a ribbed cuff style may be used.

- **Face** - a full-face visor should be used to protect eyes and face where splashing or spraying may occur and, in particular, where operations are carried out at eye level.
- **Body** - a laboratory coat or overalls should be worn at all times. Open pockets and turn-ups where liquid could collect should be avoided. Trouser bottoms should overlap boots or shoes for the same reason.
- **Feet** - sturdy shoes are recommended for handling liquid nitrogen vessels. Open-toed shoes must not be worn under any circumstances.

3.3 When freezing crystals and working with frozen crystals

- This work involves small volumes of liquid nitrogen, between 2 and 5 litres of liquid N_2.
- When freezing crystals and working with frozen crystals, care should be taken to ensure that eyes are protected at all times: safety glasses should always be worn.
- When handling crystals it is not practical to use the large cryogloves. As an alternative two pairs of laboratory gloves can be worn. Ensure that there are no holes in the gloves and replace them frequently. A thin pair of silk or cotton gloves may also be useful inside the plastic gloves.
- 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.

3.4 Filling Dewars

Filling from **a pressurized storage Dewar**, e.g. the 120 litre vessels:

- Point the hose away from face and body.
- 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.

With **non-pressurised containers**, e.g. the dispensing Dewar:

- Do not plug the entrance with any device that would interfere with the venting of gas.
- Use only the loose fitting necktube core or an approved accessory.
- Dispense slowly to avoid splashing
- Ensure that the receptacle is stable and close to the mouth of the Dewar.

3.5 Disposal of liquid nitrogen

- Liquid nitrogen should be disposed of by evaporation.
- In room 5.20, dispose of liquid N_2 by pouring it into the cardboard boxes containing pipet tip trays for recycling. The large surface ensures quick evaporation. Do not spill across floor.
- In a laboratory, the container should be left either in a fume hood with the door closed or in a well ventilated large area, with the lid off. Prop it up or leave in a secondary container if necessary to prevent it being knocked over.

- Never pour liquid nitrogen down the sink.
- Label it clearly so the cleaners know not to touch it/knock it over.

3.6 Transportation of liquid nitrogen to and from 5.20 Flowers

Transport liquid nitrogen in the goods lift (never in the passenger lift), following the steps below:

- Liquid nitrogen should only be taken into Flowers through the front entrance or the back door, which can be reached via the passageway between Flowers and SEC.
- Wear personal protective clothing, including lab coat, face mask and cryo gloves (see section 3.1 above).
- Place Dewar in lift safely. Make sure it is stable and will not topple over. Put it in a box if necessary.
- Pull the barrier across the lift doorway to prevent others from entering the lift.
- Send the lift to the appropriate floor (ground or 5th floor).
- Take the passenger lift or the stairs yourself.
- Retrieve Dewar from the lift.

4 Emergency procedures

In the event of a spillage or accidental release of a significant quantity of liquid nitrogen (if low oxygen alarm sounds), the following procedure should be followed:

- Evacuate the area. Deploy warning signs if necessary.
- Ventilate the area. Open doors and windows or activate forced ventilation to allow any spilt liquid to evaporate and the resultant gas to disperse.
- 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 the low oxygen alarm has silenced.
- Prevent liquid nitrogen from entering drains, basements, pits or any confined space where accumulation may be dangerous.

5 First aid

- Where inhalation has occurred, the victim (who may be unconscious) should be removed to a well-ventilated area.
- **Rescuers should not put themselves at risk - a contaminated area should not be entered unless considered safe.**
- The person should be kept warm and rested whilst medical attention is obtained.
- If breathing has stopped, a trained first aider should commence resuscitation.
- Where contact has occurred, the aim should be to slowly raise the temperature of the affected area back to normal.
- For minor injuries, clothing should be loosened and the person made comfortable.
- Clothing should not be pulled away from burned or frozen skin.
- Douse the affected area with copious quantities of tepid water (40°C) for at least 15 minutes, then apply a sterile burn dressing to protect the injury until the person can be taken to receive hospital treatment.
- Do not use a direct source of heat such as a radiator.
- Do not permit smoking or alcohol consumption.
- Do not give analgesics (e.g. paracetamol, aspirin).
- For major injuries apply first aid as far as is practicable and arrange for the victim to receive medical attention.
- On the wall in each lab where liquid nitrogen is used there are instructions on how to treat cold burns and how the patient should be treated in hospital. Please take

a copy of these notes, BOC Gases Guidance Note G4968 *"Treatment by medical practitioner or hospital"*, with the patient in hospital and show it to the medical staff there.

6 Training and further reading

All liquid nitrogen users should read these local rules and sign the form in the file on the left as you go into the X-ray lab 5.20, to indicate that they have read the local rules. Training is the responsibility of the department and full documentation of training should be maintained.

The Safety Unit offers periodic training in the use of cryogenic substances. The "Laboratory Gases and Decanting Liquid Nitrogen" course is recommended for those using liquid nitrogen on a day-to-day basis: More information is on the web:

http://www3.imperial.ac.uk/staffdevelopment/safety/index/gassafety2.

Also you may wish to read the college guidance notes from which much of the information in this document is drawn: Health and safety/guidance notes/guidance note 15: "storage, use and transportation of liquid nitrogen within college premises (September 2000)."

7 Risk assessment

Users are required to read the "Risk assessment for working with liquid nitrogen in room 5.20, in the Flowers building, Imperial College", make sure to agree to the points in the document and sign the training register to say that they have read it.

If you encounter any risks or hazards that are not covered by the risk assessment, you must inform the Structural Biology Facilities Manager (Andreas Förster) or the Director of the CSB (Xiaodong Zhang) at the earliest possible date so that the risk can be assessed, problems corrected and the assessment updated.

8 Relevant legislation

The *Management of Health and Safety at Work Regulations 1999* require every employer to make a suitable and sufficient risk assessment of the risks to health and safety of his employees to which they are exposed while at work. The Regulations also stipulate a requirement for the provision of adequate information, instruction and training and for procedures for dealing with serious and imminent danger. The *Personal Protective Equipment at Work Regulations 1992* require employers to provide suitable protective equipment where risk cannot be adequately controlled by other means which are equally or more effective.

The *Confined Space Regulations 1997* may also apply where unventilated or poorly ventilated areas are concerned.

In addition to the above, the *Pressure Systems Safety Regulations 2000* apply to all systems containing liquefied gas operating at a pressure greater than 0.5 bar (approx. 7 psi) above atmospheric. These Regulations require users to ensure that systems are properly maintained, periodically examined (and adequate records of examination kept) and are operated within established safe operating limits.

Note: the *Control of Substances Hazardous to Health (COSHH) Regulations* do not apply to the use of liquid nitrogen as it is not classified as a substance hazardous to health but as an asphyxiant.

9 Whom to contact

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10 About this document

This document is a modification of the college Guidance notes for use of liquid nitrogen written by John Luke. The document was modified by Liz Carpenter to consider the use of liquid nitrogen in the Flowers building for X-ray crystallography. Revision and updating of the document was completed on 8.7.2013 by Andreas Förster.