NATURAL ENVIRONMENT RESEARCH COUNCIL

NERC GUIDANCE ON SAFE USE OF CRYOGENICS

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BACKGOUND

This guidance document provides information and advice on the hazards that may arise and suitable precautions that may be necessary when using, storing, and transporting low temperature liquefied or solidified gases (commonly referred to as cryogenics). The risks from use of cryogenics are considerable and have led to deaths in laboratory situations from asphyxiation due to oxygen displacement.

DEFINITIONS

Cryogenic liquid – A liquid produced from a gas that can be liquefied, and in some cases solidified, by the application of pressure and cooling within the temperature range -75^{o}C to -270^{o}C. Examples of materials likely to be encountered within a research environment, with associated boiling points at atmospheric pressure, are Nitrogen (-196^{o}C), Oxygen (-183^{o}C), Helium (-269^{o}C) and Carbon Dioxide (sublimes at -78.5^{o}C).

Dewar – A vacuum insulated container for storing very low temperature liquid gases**.** Sometimes dewars are considered to only include small open (non pressurised) vessels but in most laboratory situations portable closed pressure vessels with pressure bleed/relief to allow escape of evaporating gas are also called dewars. Larger fixed bulk pressure storage vessels are normally termed 'tanks'.

Dry Shippers - dry shippers contain a cryo-absorbent material in the inner wall which soaks up the liquid nitrogen so there is no free liquid present and holds samples in a vapour phase environment at cryogenic temperatures. This means no spillage of liquid nitrogen will occur during transport and shipping.

Dry Ice – solid carbon dioxide (CO_2) also known as 'cardice'. This is a solid cryogen rather than a cryogenic liquid and sublimes at -78.5^{o}C.

HAZARDS FROM CRYOGENIC MATERIALS

The hazards associated with low temperature liquefied or solidified gases mainly arise from their physical properties. They are:

- Asphyxiation - Rapidly evaporating gases can reduce the oxygen concentration of air by displacement so that it reaches dangerous levels (see table below). Areas with oxygen concentrations below 18% must never be entered. It is recommended that oxygen alarms are set to alarm at 19%
- Cold burns, frostbite and hypothermia from contact with liquefied/solid materials, cold surfaces or gases

- Over pressurisation if the large volume expansion caused by the liquid becoming a gas is confined or trapped
- Fire from oxygen enriched atmospheres generated by the condensation of oxygen onto surfaces
- Materials becoming brittle from the effects of extreme cold and could result in catastrophic failure
- Manual handling risks from delivering/transporting of cryogenic materials and their containers around site may create manual handling hazards.

Effects of lowered oxygen levels

Oxygen content of air	Signs and symptoms of asphyxia
18% - 19%	May affect physical and intellectual performance without person's knowledge.
15% - 18%	Decreased ability to work strenuously. May impair co-ordination and induce symptoms in persons with coronary, pulmonary or circulatory problems.
12% - 15%	Respiration deeper, increased pulse rate and impaired co-ordination, perception and judgement.
10% - 12%	Further increase in rate and depth of respiration, further increase in pulse rate, performance failure, giddiness, poor judgement, cyanosis (blue lips).
8% - 10%	Mental failure, nausea, vomiting, fainting, ashen face, cyanosis.
6% - 8%	Loss of consciousness within a few minutes, resuscitation possible if carried out immediately.
0% - 6%	Loss of consciousness almost immediate, death ensues, brain damage even if rescued.

Effects of CO_2 enrichment

CO_2 poses an intoxication risk which is actually more serious than asphyxiation and occurs at lower displacement levels. CO_2 has been assigned a Workplace Exposure Limit (WEL) of 0.5% (5000 ppm) averaged over an 8 hour working period of 1.5% (15,000 ppm) averaged over a 15 minute short term exposure.

Dangerous conditions with elevated CO_2 levels cannot be detected by a low oxygen alarm alone. CO_2 forms a key part of the biochemical mechanism involved in the breathing reflex and high levels act as an intoxicant or narcotic which depresses and eventually suppresses breathing. Inhaling levels above 10% CO_2 will rapidly lead to death, which will occur even if there is an adequate level of oxygen to support life.

CO_2 in air	Signs and symptoms of narcosis
1% (10,000 ppm)	Slight symptoms; possible increase in breathing rate
2% (20,000 ppm)	Breathing becomes deeper – 50% above normal
3% (30,000 ppm)	Laboured breathing – 100% above normal, increased pulse rate, reduced hearing, headaches
4% – 5% (40,000 ppm – 50,000 ppm)	As for 3%, but after 30 minutes exposure signs of poisoning evident with a choking sensation

5% - 10% (50,000 ppm – 100,000 ppm)	Characteristic sharp acidic smell apparent, heavily laboured breathing, ringing in ears, visual disturbances, loss of consciousness within minutes
> 10% (> 100,000 ppm)	Rapid loss of consciousness with risk of respiratory arrest and death, concentrations > 20% are immediately life threatening

Metal surfaces in contact with liquid nitrogen can generate oxygen enrichment as liquid nitrogen is at a temperature below the boiling point of oxygen so may allow it to condense out of the atmosphere. If there is any possibility of collection or enrichment then precautions may be required eg threads on pipework must be kept clean and free from grease and fitted with PTFE sealing tape.

The degree of risk will vary according to the location of use (eg levels of ventilation), the specific material, the delivery container, the volume and pressures within containers.

Risk assessment will be necessary to cover activities involving the use, storage or transportation of cryogenic materials. The following sections suggest controls to reduce risk from the handling of cryogenic materials to an acceptable level.

STORAGE OF BULK QUANTITIES OF DRY ICE

Dry ice can be purchased or produced in a solid, powder or pellet form. Supply as a solid block should be avoided as its use will require breaking up into smaller usable sized pieces which will give a risk of generating flying particles. Dry ice will usually be delivered in bags and can be kept in specially insulated storage chests. Excess material or residues of dry ice should left in a safe place, ideally in the open air or in a very well ventilated position such as a fume cupboard, to safely evaporate. Much condensation may be generated during this process. Dry ice should not be disposed of in sinks as this can cause failure of the drain due to extremely low temperatures and allow accumulation of CO_2 in drains.

Dry ice should ideally be stored in specially designed insulated trunks or chests which can reduce sublimation to about 2% per day depending on how full they are kept. When collecting dry ice from the chest care the user must take care not to place their head inside the chest or breathe its internal atmosphere as it will be contain high levels of CO_2, even if it is nominally empty.

The best place to locate a dry ice storage chest is outdoors in a well ventilated location. Where the dry ice storage chest is located within a building the storage area must be sufficiently well ventilated to adequately dilute releases of gaseous CO_2. Remember the atmospheric levels of CO_2 sufficient to cause risk are well below those of other non-toxic cryogenic gases and an oxygen meter would be insufficient to warn of the hazard.

Dry ice should not be put in an unventilated storeroom or freezer/cold room or any other enclosed space that may be entered as it will rapidly evaporate to generate an unsafe atmosphere.

Dry ice should not be placed directly in a conventional fridge or freezer as it may damage the device due to the extreme low temperatures.

It is practice in some areas to place spare/unused dry ice in -80º C freezers in order to preserve stocks for longer periods of time. This is to be discouraged as even though the temperature will theoretically be below the sublimation temperature CO_2 will still be generated and create an unsafe atmosphere. A -80º C chest freezer will act as a trap to hold and retain any evaporated CO_2 so if a person's head enters and breathes the inside atmosphere this could lead to immediate loss of consciousness and falling into the chest. If only infrequent use of relatively small quantities of dry ice is required, consideration should be given to obtaining a dry ice maker.

If it can be justified as being essential that dry ice is stored in a -80º C freezer, an upright style, which does not require the user's head to enter its interior and whose vertically hinged door allows safe escape of trapped CO_2 at low level when opened, should be used. In addition, stringent precautions such as keeping the freezer locked to prevent unauthorised access and suitable signage should be in place.

STORAGE OF BULK QUANTITIES OF CRYOGENIC LIQUIDS

Static bulk storage tanks are often used for cryogenic liquids at sites where there is large usage. The location for bulk storage tanks of cryogenic liquids should be in a safe, well-ventilated and secure location, preferably in the open air. For smaller bulk storage vessels located in the open air, it may be desirable for them to be provided with simple roof cover for protection from the weather and direct sunlight. Tanks of greater than 500l capacity must be located in the open air.

Choice of location for bulk storage vessels should take into account:

- The size of the tank
- Nature of surface on which tank is located, especially if liquid oxygen is stored
- Space/clearance for installation and maintenance of the tank and its fittings
- Access for filling or dispensing from the tank
- Clearance distances to ensure ventilation/dispersal of vapours, protect neighbouring activities and protect against build-up of unsafe atmospheres eg away from basements or pits
- Protection from fire and impact from vehicles etc.
- Security to prevent unauthorised access/interference

Bulk storage tanks are pressure vessels and should be subject to a written scheme of examination which specifies the nature and frequency of planned preventative maintenance, the safety provisions to prevent over-pressurisation and any routine inspections necessary. Large bulk storage vessels in the open air should be kept clean and painted white to minimise solar heat gain.

Bulk storage containers of liquid cryogenic liquids should be located on impervious concrete surfaces rather than tarmac. This is especially important for liquid oxygen as contact with hydrocarbons can lead to spontaneous ignition. The areas should be free from drains as a spillage or leak of a cryogenic material may cause cracks should it enter drainage.

Signs to warn of the dangers from ‘Extreme cold’ should be clearly displayed, especially where there is a possibility of contact with cold surfaces as this is more likely to result in burns than direct contact with the gas.

Pressurised dewars must be dedicated to one type of gas unless they have been re-certified for another gas by the manufacturers.

Ice may form on necks of dewars (although not if the correct dewar stopper is fitted), on exposed pipework and vents or pressure reliefs of pressurised dewars. Normally this is not a problem but on rare occasions the build-up is extreme and a plug forms that blocks an essential safety component. This may lead to build up of internal pressure and may result in ejection of the plug under force or even, in extreme cases, failure of the vessel. Expert help should be sought before attempting to deal with such a condition.

FILLING MOBILE PRESSURISED DEWARS, DRY SHIPPERS, NON-PRESSURISED DEWARS AND FLASKS

When filling or dispensing cryogen at pressurised dewars, ensure the bursting disc or pressure relief valve is not pointed towards the face.

An inspection of the container to ensure no leakage is occurring and the container is sound should be made prior to filling operations. Where damage is suspected the container should not be used and defects reported.

Hoses should be regularly checked to ensure they are not subject to kinking and the braiding is not damaged. These may be indications of internal damage and if identified hoses should be replaced. Even where no specific damage is identified hoses should be replaced every 5 years.

Filling of cryostats, dewars and flasks frequently results in vapour clouds and ‘spitting’ of boiling liquid and spillage. Where possible measures to prevent overfilling should be taken e.g. dip sticks used to measure the level in the vessel. Other methods include use of phase separators in the end of the filling hose to condense the liquid and slow filling to reduce the amount of splashing and vapour generated.

Significant noise levels, above 90 dB (A), can be experienced during filling mobile pressurised dewars from a bulk storage tank. Silencers can be used to mitigate the risk from noise. Where these are not available ear defenders should be used.

TRANSPORT OF CRYOGENIC LIQUIDS

Transport of cryogenic material in poorly ventilated spaces, such as lifts, could present a problem should a spill occur or the lift break down. The following points require attention:

- the load should travel in the lift unaccompanied

- lifts used should ideally have a key control over-ride for the operator who can send the lift to a designated floor and, if they have no-one to receive it at the destination, use the stairs to meet it without anyone being able to call or operate the lift
- where there is no key control over-ride, the lift should be commandeered for the duration of the transportation, ideally with a barrier and signage placed in the lift car across the door in front of the dewar to indicate that the lift is being used to transport cryogenic material and must not be entered. This is likely to require a two person operation - one on the despatch floor, the other on the receiving floor.

Personnel transporting containers around site should have appropriate training on both the hazards and precautions associated with handling cryogenic material and manual handling of loads. Lone working and manual handling issues should be taken into account.

Transportable dewars should not be taken down steps or stairs. Slopes should be avoided if at all possible. Floor surfaces that dewars are transported across should be in good condition, smooth and even without holes or depressions such as floor drains. All areas through which dewars are transported should be well ventilated and have sufficient space/width to not impede the workers or any other staff who may work in that area. Two persons may be necessary to transport dewars to minimise manual handling issues and possibility of spills. Densely populated areas should be avoided.

If collecting and transporting material that needs to be kept frozen at liquid nitrogen temperatures, the use of dry shippers should be considered. Dry shippers eliminate the potential for spillage during transit by absorbing the liquid nitrogen into a carrier material, effectively keeping the sample in the gaseous phase. Dry shippers which have been correctly charged contain no free liquid nitrogen and are not considered as Dangerous Goods (DG) for any mode of transport provided the material being transported within them is not a DG eg is a non-infectious biological sample. However, liquid nitrogen itself is a DG for all transport modes although there are exemptions. If off-site transportation of liquid nitrogen cannot be avoided, advice from a competent person must be sought eg the NERC Dangerous Goods Safety Advisor (DGSA).

Loads containing cryogenic liquids taken in vehicles should be transported in a separate compartment segregated from the driver and any passengers (the boot of a normal saloon or hatchback should not be considered a separate compartment). Where this is not possible, the quantity must be kept to a minimum, the driver/passenger compartment kept well ventilated with one or more windows open, care taken to ensure it is secured to prevent spillage and the package is appropriately labelled. Taking a personal alarmed oxygen meter if the cryogen preserved samples are not in a separate compartment of the vehicle is strongly recommended

TRANSPORT OF SAMPLES STORED IN DRY ICE

Materials required to be kept frozen are often transported using dry ice to maintain the temperature of the samples. The material sublimes to a narcotic gas and should therefore not be carried in poorly ventilated areas. The advice on taking dry ice in a vehicle is the same as given above for a cryogenic liquid except that it is very strongly recommended a CO_2 personal alarmed meter is taken in the vehicle if the dry ice is not in a separate compartment; a low oxygen alarm alone would not give warning of dangerous levels of CO_2 (infra red detectors are best for this application).

Do not seal dry ice in closed vessels as there is potential for generation of pressure and ultimately explosion. The expansion factor of dry ice from solid to gas is 554 and the pressure at which CO_2 may be liquefied by pressure alone is above 5.1 bar. Therefore, unless the sealed vessel is able to withstand such a pressure it will fail, possibly catastrophically, with the sudden release of large amounts of energy.

Dry ice is regarded as a DG for shipment by air but not under UK road transport regulations. Appropriate UN approved packagings which are suitably vented and insulated should still be used for road transport. For transport by air of dry ice, the outer packaging **must** carry a Class 9 transport diamond (safety sign for miscellaneous dangerous goods), be labelled with the letters and numbers "UN1845" (the UN number for dry ice) and close by the words "DRY ICE" in capital letters, and the net weight in kg of solid CO2 (on filling!). However, many road transport couriers will make a similar stipulation for packages containing dry ice so this is best practice for all shipments.

Always unload the material as soon as possible at the end of the journey to a suitable, well ventilated, storage location. Do not place dry ice in an unventilated cold store. The dry ice in an approved insulated shipper will last approximately three days from initial filling.

The driver must be aware of the symptoms of CO_2 intoxication and what emergency actions are required when transporting dry ice.

INSTALLATIONS FOR USE OF CRYOGENICS

It is possible to postulate a number of types of installation for supply and use of cryogenic liquids in laboratory situations. Where possible it is best to install a bulk cryogen storage vessel or dewar outside the building in a safe accessible position in the open air and pipe the cryogenic liquid in to the point of use within the building. This can be done via specially designed insulated pipework known as Super Insulated Vacuum Lines (SIVL). This type of installation eliminates the generation of vapours within the working room during filling operations, the risk of major spills within the building, reduces the distance dewars need to be transported and eliminates this activity within buildings as well as use of lifts. There are practical limitations on the distance of SIVL can travel but care in the initial design of the facility may assist.

The British Compressed Gases Association has recently published new guidance on biostores that may be of use here (see references).

SAFE USE OF CRYOGENIC LIQUIDS

Non-pressurised storage vessels dewars/flasks and cryostats must be designed for this application, dedicated to one specific gas and appropriately labelled. The vessels must be regularly examined for signs of damage or deterioration, maintained as necessary and have an appropriate top to prevent the ingress of water.

Domestic vacuum flasks must not be used to contain cryogenic liquids. They are not designed for such low temperatures and may be inadvertently sealed. The cryogen may shatter the ordinary glass flask or cause the rubber seal to fail and allow leakage into the cavity between the glass vacuum liner and the outer flask wall, leading to an explosion as the liquid rapidly evaporates in a confined space.

The floor/bench surface in areas used for the storage/use of cryogenic materials must be suitable to withstand the effects of freeze/thawing as there will inevitable be small spillages in use. Normal laboratory vinyl flooring is not resistant to these effects (although very heavy grades may have better resistance to cracking) but concrete, steel or epoxy resin flooring is resistant to cold spills. Rooms used with liquid nitrogen should have vision panels so it is possible to see inside without entering eg should any alarms be triggered.

The potential for asphyxiation arises when gases evaporate during use and are not removed or diluted. The oxygen levels in an enclosed area used to handle or store cryogens will depend on the volumes handled, the likely rate of release, spillages or leaks, the volume of the area, the nature/location of the ventilation combined with the air change rate achieved and localised features such as pits or areas with restricted air exchange.

The asphyxiation risks can be divided into **sudden**, eg where there is a spill resulting in rapid generation of high levels of vapour when even well ventilated areas may not be safe, and **gradual** where slow release of vapours may reduce oxygen levels to an unsafe level. Precautions should be designed to cope with both eventualities. Formulae to allow calculation of likely oxygen concentrations for both situations are given at Appendix 1 and will help in selection of suitable precautions.

High air change rates should be provided in any area where cryogenic materials are used. Natural ventilation is unlikely to provide sufficient ventilation for most situations involving cryogenics unless the operation is being undertaken in the open air. A naturally ventilated room may only give an air change rate of 1x an hour or less if the windows are well sealed. In a very well designed naturally ventilated room with permanent large openings to outside atmosphere on opposite sides comprising a total of 1% - 3% of the wall area it may be possible to gain 5 air changes per hour. Basements pose particular issues if reliance is made on natural ventilation as their air change rates likely to be significantly lower than areas at ground level or above.

At least 10x air changes are ideally required for situations involving use or storage of cryogenic liquids. This means that in most internal areas forced ventilation will be required which should include low level extract, as the cold vapours initially generated will be heavier than air, as well as ceiling extract (especially if liquid helium is present) and suitable fresh air make-up. The normal forced ventilation rates may need to be supplemented by emergency ventilation to give even higher air change rates of up to 20x or 30x an hour to cope with spillages or quenches. This can be triggered by interlocking to the low oxygen alarm. Situations where normal air change rates may be reduced should be taken into account in any calculations eg power failure or 'night setback' where there is reduction of air change rates out of normal working hours.

Low oxygen alarms need be installed in workplaces where there is a potential for depletion of oxygen. Such areas will include rooms where large volumes of cryogens relative to the volume of the room and its ventilation rate are dispensed, used or stored and rooms where there is equipment with the potential to generate large volumes of vapours from cryogens in a short space of time (eg those containing Nuclear Magnetic Resonance or Magnetic Resonance Imaging machines where 'quenches' may occur).

The sensors for low oxygen alarms should ideally be placed at a height of between 1 and 1.5 m from the floor. They can be supplemented by other sensors at a higher level. Location of oxygen sensors at low levels close to the floor should be avoided. These are liable to give rise to unwanted alarms which create complacency if triggered frequently when there is little risk of asphyxiation eg as slugs of cold vapour are momentarily released close to floor level during filling or dispensing operations.

Oxygen alarms should be set to trigger if the oxygen concentration drops below 19%. The alarm should give a visible (ie illuminated such as with a beacon) and audible signal both inside and outside the room. The sensors must be subject to at least annual checking, replacement as necessary and calibration. In event of the alarms being activated all personnel must evacuate the room immediately. Signs should be placed inside the room requiring it is left immediately when the alarm sounds and not re-entered until the alarm has been investigated and the area declared safe by a competent, responsible person. Signs must also be displayed outside the entrance to the room indicating there must be no entry if the alarm is illuminated/sounding. Rooms with low oxygen alarms should have hazard warning signs displayed outside entrances to warn of the presence of cryogens and the potential for oxygen depletion. If a two stage oxygen alarm system is installed, the first stage 'alert' alarm may be set at 19.5% and the second stage 'evacuate' alarm set at 18.5%. With a very well designed and highly engineered system it may also be possible to link the low oxygen alarm system to a door lock to prevent unauthorised access (although egress from within the room must not be prevented) and to shut off supply of liquid cryogen from outside the room.

The possibility of lone working or working out of normal working when reduced numbers of persons are available to provide assistance should be taken into account when considering precautions for use of cryogenics.

Good sample management systems should be in place for storage in liquid nitrogen freezers. This will not only reduce the time taken to locate and retrieve the correct, desired sample but also reduce exposure to liquid nitrogen and generation of vapours. It will also aid other safety aspects such as biosafety and inventory control. Vial lifters are useful aids. Vapour phase freezers are preferred to liquid phase designs. Where samples are stored in liquid phase in screw capped containers, the containers should have a seal around the outer edge of the cap to prevent liquid seeping into the screw thread which can cause the cap to be forced off with explosive force as the liquid cryogen rapidly warms up and evaporates. All sample containers should be immediately placed in a fracture-proof secondary container (not glass) after retrieval and during transport, being retained there until thawed.

All staff working with cryogenic materials will need training on the hazards and precautions required to help establish competence. This should cover as a minimum:

- Cryogen hazards
- General safety precautions relevant to this work eg alarms, ventilation systems
- Emergency procedures
- Use of PPE
- First aid
- Any other relevant special procedures

Liquid nitrogen has the potential to condense oxygen from the atmosphere which if it comes into contact with oil or grease can give rise to fire.

Areas and equipment which may have extremely cold surfaces due to the presence of cryogenic material can be labelled ‘Caution: Extreme Cold’ accompanied by the ‘snowflake’ symbol.

Equipment such as Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imagining (MRI) use both liquid nitrogen and liquid helium to maintain the temperature of the magnet. Liquid nitrogen is slowly evaporated as it helps maintain the levels of liquid helium around the magnet. Failure of the equipment may result in a ‘quench’ when the liquid helium within the magnet boils off rapidly, followed by the liquid nitrogen. Low oxygen alarms are normally essential for such installations but may also be linked to an emergency ventilation system which is triggered by the low oxygen alarm to increase extraction rates, ideally as close as possible to the vent points. When the magnets in NMRs and MRIs are brought down to operating temperatures there will be very large volumes of cryogen evaporated so it is essential that all ventilation systems and alarms are fully operational before this is attempted.

Slush or cooling baths are frequently used to control reactions or condense products via cooling loops. Slush baths are prepared by combining materials to generate the desired temperature and range from ice and salt mixtures to solvent and cryogenic mixtures. The following table lists the temperatures of some of the common slush bath compositions but many others are available:

Solvent / additive	**Temperature (^{0}C)**
Ice / salt (3 : 1)	- 8
Carbon tetrachloride / dry ice	- 23
Acetonitrile / dry ice	- 44
Chloroform / dry ice	- 61

Ethanol / dry ice	- 72
Acetone / dry ice	- 78
Hexane / liquid N_2	- 94
Ethanol / liquid N_2	- 116

Fires have been experienced when the cryogen in the slush bath has all evaporated and the solvents they contain are at room temperature as the flammability of the solvent is then much higher than when they are at their operating temperature.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Appropriate PPE should be provided to protect hands, face and body. Possible PPE includes:

- safety glasses, goggles or visor (the latter being preferred, with chin and brow protection if possible)
- non-absorbent insulated gloves which either have long or elasticated cuffs to cover wrists and prevent trapping of spilt cryogen
- cryogenic apron
- overalls, preferably of a non woven fabric to avoid liquid penetration,
- safety footwear
- hearing protection, where necessary, when filling or dispensing into dewars from bulk pressurised vessels.

There is no specification for the eye protection to be used when handling cryogenic materials but those specified for use with molten metals may offer better resistance to extreme thermal shock. Polycarbonate is often used. Safety glasses (which only provide protection to the eyes), goggles and visors should be replaced if they have been directly splashed with cryogenic materials.

It is important that splashes of cryogenic liquid are not be trapped against the body. Pockets or other means of channelling/containing the liquid should be avoided as should turn-ups on trousers. Sleeves should be pulled down over wrists if shorter gloves are worn and trouser hems worn over the tops of safety shoes or boots not tucked in.

EMERGENCY ACTION

If eyes or skin come into contact with cryogenic materials or very cold surfaces, do not attempt to remove frozen clothing or free hands or limbs by forceful action as this will remove layers of skin. The temperature of the affected area should be raised gradually by immersing the affected area in tepid water. Medical assistance should be sought immediately.

Oxygen deficiency or high CO_2 can lead to loss of awareness, distortion of judgement or suppression of breathing. As the oxygen levels fall fainting, brain damage and death ensue. Any person suffering the effects of oxygen deficiency should be removed to open air, provided the rescuer does not place himself at risk. Self contained breathing apparatus is essential to enter areas where there is a likelihood of oxygen deficiency and no attempt at rescue should be attempted without assistance from competent persons.

Where there is possibility of localised enrichment of oxygen, try to stop release, switch off electrical appliances and extinguish naked lights. Evacuate area and ensure adequate ventilation. Where a person's clothes have caught fire they should be deluged with water and removed to fresh air.

REFERENCES

1. British Compressed Gases Association Technical Information Sheet TIS No 7 (rev 1, 2010): 'Guidelines for the Safe Transportation, Storage, Use and Disposal of Dry Ice Products'.

2. British Compressed Gases Association CP27 (rev 1, 2004): 'Transportable Vacuum Insulated Containers of not more than 1000 litres volume'.

3. British Compressed Gases Association CP36 (rev 1, 2011): 'Cryogenic Liquid Storage at Users' Premises'.

4. British Compressed Gases Association CP30 (rev 1, 2008): 'The Safe Use of Liquid Nitrogen Dewars up to 50 litres'.

5. British Compressed Gases Association CP25 (rev 2, 2004): 'Revalidation of cryogenic static storage tanks'.

6. British Compressed Gases Association Guidance Note GN 19 (2012) 'Cryogenic Sample Storage Systems (Biostores) Guidance on Design and Operation'.

7. Medical Research Council and CryoService publication (2010): 'Standards for Liquid Nitrogen Supply: Systems for Life Science Applications', available from CryoService, Warndon Business Park, Worcester, WR4 9RH.

APPENDIX 1 – CALCULATIONS FOR OXYGEN LEVELS WHEN STORING, TRANSPORTING OR USING LIQUID NITROGEN

A calculation based on the formula to calculate the resulting oxygen concentration should be undertaken prior to deciding to site a vessel containing liquid nitrogen (although figures to allow calculations for other cryogenic liquids are given) in a workplace. The rate of air changes can be taken into account but situations when these rates fluctuate, eg power failure or reduction of air changes over night if applicable, must be taken into account.

Formula to apply for sudden releases of liquid nitrogen (eg spillage) where dilution from ventilation is not applicable:

Oxygen (O_2) Concentration % = $$\frac{V_o}{V_w} \times 100$$

Where:

V_o = volume of oxygen (m^3)
V_w = volume of available air (m^3), not including above 2m in height

$V_o = 0.21 \times [V_w - (V_t \times \text{the expansion factor of cryogenic liquid})]$

V_t = net tank capacity (m^3)

Liquid nitrogen has an expansion factor (liquid to gas) of 683.

Note: Expansion factors of other liquid cryogens are: oxygen 860, helium 754 and argon 840. Although this calculation cannot be used for dry ice in this format, the expansion factor of CO_2 from solid to gas is 554.

$V_w = V_r - V_i$

V_r = volume of workplace obtained from room/area dimensions: length x width x height (height only up to a maximum of 2 metres).

V_i = volume occupied by objects/furniture/equipment in room.

If the calculation shows the oxygen concentration will be less than 19.5%, then an action plan will need to be devised and implemented to ensure adequate oxygen levels can be maintained.

Worked example for spillage

A room 4m long x 5m wide x 2.5m high located at ground level houses a large 50 litre (0.05 m^3) unpressurised dewar containing liquid nitrogen and has fixed benches and cupboards with doors beneath measuring 6m long x 0.9 m tall and 0.8m deep but no other ‘enclosed’ furniture or large items of equipment.

V_w = (4 x 5 x 2 - *2m is used as max room height)* – (6 x 0.9 x 0.8) = 40 – 4.32 = 35.68 m^3

$V_o = 0.21 \times [35.68 - (0.05 \times 683)] = 0.32\ m^3$

$$\% O_2 = \frac{0.32 \times 100}{35.68} = 0.9$$

which means there would a serious problem if the whole dewar was spilt as the O_2 in the room would be almost completely displaced by N_2.

<u>Formula to apply to a storage location where ventilation may be taken into account to allow for gradual release</u>

$$\mathbf{Ct = L \div (V \times n)}$$

Where:

Ct = % gas concentration,
V = room volume (with height above 2m discounted),
n = air changes per hour and
L = gas release (m^3 per hour)

Worked example for gradual release

A room 5m long x 4m wide x 2.5m high located at ground level with one air change per hour (ie very poorly ventilated) houses a large 200 litre pressurised dewar containing liquid nitrogen. The dewar manufacturer quotes an evaporation rate of 4 litres per 24 hours. As the manufacturer's quoted evaporation is for a new dewar and insulation deteriorates over time, this evaporation rate is multiplied by two.

$$L = \frac{8 \times 683}{24 \times 1000} = 0.228\ m^3 \text{ hour}$$

Volume of room = 5 x 4 x 2 = 40 m^3 (*note: height used in calculation is 2m not actual room height of 2.5m)* and no of air changes is 1

$$Ct = \frac{0.228}{40 \times 1} = 0.0057 = 0.57\%$$

This means the room O_2 concentration will be 21 - 0.57 ≈ 20.4% which is not a problem.

<u>Formula to apply to a location where loss during filling operations needs to be taken into account</u>

It is normally assumed that up to 10% of the fill may be lost during filling.

$$\mathbf{V_o = 0.21 \times \left(V_R - \left(\frac{0.1 \times V_D \times f_g}{1000}\right)\right)}$$

Where:

V_o = volume of oxygen in the room in m^3
V_R = volume of room in m^3
V_D = capacity of dewar in litres
f_g = expansion factor

Worked example for loss during filling:

Volume of dewar is 30 litres, cryogenic liquid is nitrogen, volume of room (up to 2m height) is 40 m^3 :

$$V_o = 0.21 \times \left(40 - \left(\frac{0.1 \times 30 \times 683}{1000}\right)\right)$$

$= 7.97\ m^3$

The concentration of oxygen in the room immediately after filling would be (7.97 ÷ 40) x 100 ≈ 20% which means there would be no problem.

This formula can be modified to take into account the effect of loss during filling followed by a 100% spill. In this case the 0.1 multiplier applied to represent a 10% loss of fill (in the worked example 3 litres) is increased to take account of the full volume of the dewar ie becomes 1.1 (30 litres total volume of dewar + 10% loss during filling).