Measure of the Director of the central European Institute of Technology of Masaryk University

No. 3/2023

Working Safely with Liquid Nitrogen and Other Cryogens

(effective as of 1. 6. 2023)

Pursuant to Article 7(4) of the Rules of Organisation of the Central European Institute of Technology of Masaryk University and pursuant to the provisions of Sections 101, 102, 103, 108 of Act No. 262/2006 Coll., The labour Code, as amended (hereinafter the "Labour Code"); and pursuant to the Chancellor's Guideline No. 10/2009 on the Organization of Occupational safety and Health Protection at Masaryk University, I hereby issue the following Measure:

Article 1
Subject Matter

(1) This Measure of the Director of CEITEC MU (hereinafter the "Measure") is issued to ensure safe work with liquid nitrogen and other cryogens at all departments of the central European Institute of Technology, Masaryk University (hereinafter "CEITEC MU") that work with these substances.

(2) This Measure specifies the safety guidelines that must be followed by persons handling liquid nitrogen and other cryogens.

Article 2
Definition

(1) Cryogenic liquids are substances that are gaseous at normal temperature and pressure. However, when cooled to a very low temperature they become liquids. Their boiling point is usually below -150°C. The vapours and gases which escape from these liquids are very cold. They often condense moist air to form a very dense mist. Individual cryogens become liquids under different thermal conditions and at different pressures, but what they all have in common is that they are extremely cold and that even small amounts of them can produce large volumes of gas. Anyone working with cryogens must be aware of their hazardous nature and know how to handle them safely. Each cryogenics liquid has its own specific properties, but most cryogens fall into one of these groups:

a) Inert gases: inert gases do not chemically react with other substances. They do not burn nor explode. Examples are nitrogen, helium neon, argon and krypton.
b) Flammable gases: some cryogens produce a gas that is flammable in air. The most common examples are hydrogen, methane, carbon monoxide and liquefied natural gas.

c) Oxygen: many substances considered non-explosive can burn in the presence of liquid oxygen. Organic substances can react explosively with oxygen. Thus, the hazards precautions for handling liquid oxygen must be followed separately from other cryogens.

(2) Nitrogen is an inert, non-toxic, non-flammable, chemically little reactive, colourless and odourless gas that makes up 78% of the Earth's atmosphere. It is one of the biogenic elements that constitute the basic building blocks of living matter.

Article 3

Properties of Nitrogen

(1) Properties:
d) odourless,
e) non-flammable,
f) non-toxic,
g) during evaporation, it produces a cold mist that spreads into the surrounding area,
h) the nitrogen mist falls quickly to the ground.

(2) At ambient temperature (20°C), one litre of liquid nitrogen produces approximately 700 litres of nitrogen gas.

(3) Deeply cooled liquefied gases are hazardous substances because of their physical properties and because of their physiological impact on human body. The risk stems mainly from their low boiling point at normal pressure (-196°C) and low evaporative heat, which is approximately 10 times lower than that of water.

(4) When the human body comes into direct contact with these liquids, the affected parts are rapidly cooled to well below freezing. Freezing is painless, but after thawing it is very painful and leads to shock. Also, deeply hypothermic parts of the equipment can cause freezing and skin detachment. Even inhaling cold gases alone can cause serious respiratory damage.

(5) Deeply cooled gases spilt or released freely into the open air evaporate very intensely due to their low evaporative heat and the resulting fog clouds can be dangerous, especially in adverse climatic conditions.

Article 4

Cryogenic Liquid Containers

(1) There are several types of cryogenic liquid containers, they are of different sizes and can store cryogenic liquids for different periods of time. The following points indicate the most common types of these containers.

(2) Dewar laboratory transport containers are intended only for short-term storage of cryogenic liquids, for a few days at most. They consist of a vacuum jacketed cryogenic container, usually made of silvered glass, with an outer jacket of aluminium or steel. A loose-fitting stopper-style closure is used to release the vaporized gases so that the pressure inside does not exceed the atmospheric pressure, while keeping air out to

prevent condensation of liquid oxygen. Generally, these containers are small and easy to transport. The smallest ones can be carried by means of handles, while larger models may be fitted with wheels attached to the bottom, or a trolley of appropriate size may be required. The vacuum space around the cryogen container is equipped with a pressure valve in case the inner container bursts and cryogen enters the vacuum space. In this case, pressure could grow inside the vacuum compartment and the container could burst, so it is safely drained through a safety valve instead.

(3) Before using low-pressure containers, the outer shell must be checked for corrosion. The safety valve must be visually intact. The lock cover should be easy to close without jamming.

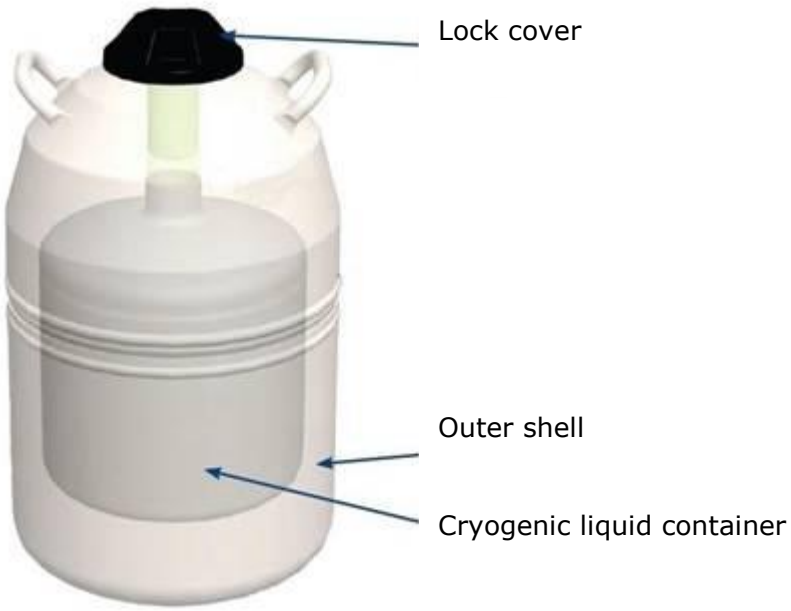


Figure 1 – Low-presure Dewar container

(4) Large cryogenic liquid storage containers consist of an inner and an outer vessel with a vacuum superinsulation between them.

(5) Small vessels (see Figure 2) are designed to hold cryogen for a relatively short period of time for various laboratory operations. Many of these boxes are equipped with a foam, loose-fitting or vented lid that can reduce the rate of evaporation of the cryogen inside the container without increasing the pressure inside the container. Depending on whether the container is fitted with a lid, the type of lid and the size of the container, they can hold liquid cryogen for several hours to several days. They are made of double-layer glass with evacuation between the layers and with a protective aluminium or steel cover. They are the most fragile of all cryogenic liquid containers, but are unlikely to implode without impact or damage to the glass. When they do shatter,

however, the process is quite vigorous and fragments of glass can be ejected at high velocity from the top opening.

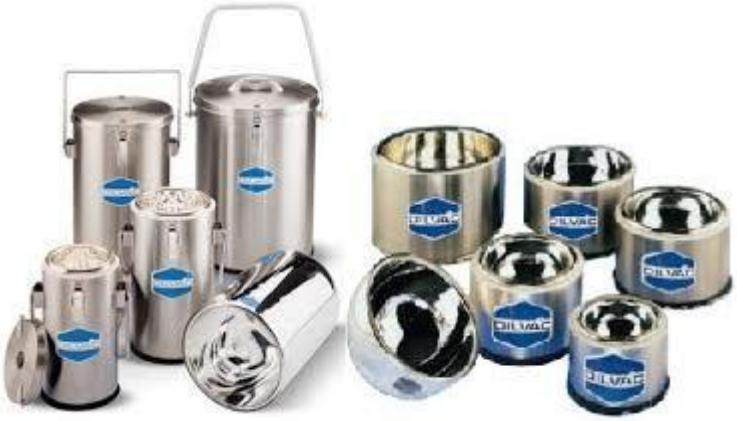


Figure 2 – Small containers for cryogens

(6) A polystyrene foam container (with or without lid) can also be used for handling and short-term storage of liquid nitrogen, see Figure 3.

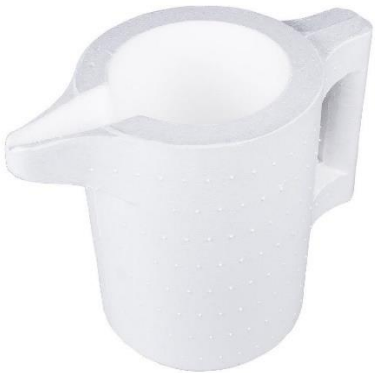


Figure 3 – Polystyrene foam container for liquid nitrogen handling

(7) Large stainless steel cryogenic liquid containers (Apollo type, etc.), see Figure 4, with vacuum insulation are used for storage or pumping of larger volumes of liquid nitrogen. They are usually equipped with wheeled runners with handles, a pumping device,

safety valves, vacuum valves, pressure and/or level gauge. Instructions for working, manipulation and storage of stainless pressure containers are provided in Article 5.

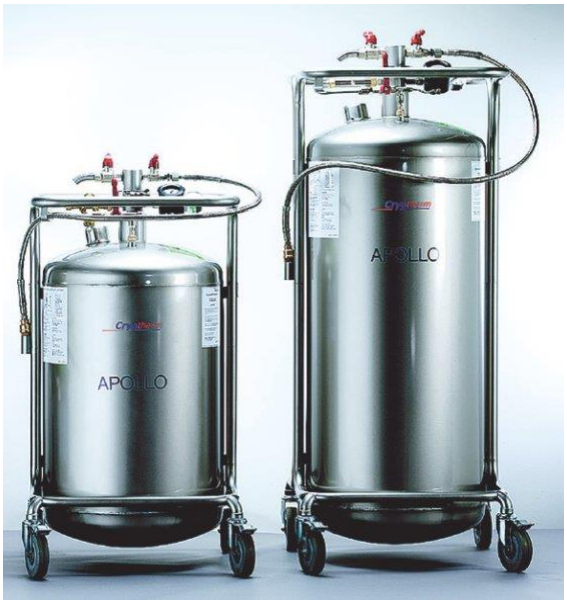


Figure 4 – Large vessels for cryogenic liquids

(8) During use, no ice should form on the outside of the aluminium cover or on the cap. If ice forms on the outside of the container, it means that the container may have lost vacuum and is no longer insulating enough to store cryogenic liquid. If there are cracks in the inner silvered glass container, liquid cryogen may enter the vacuum space and when the container is heated, the entire container may explode due to the expanding cryogen.

(9) If there is any sign of damage or loss of insulation, the container must be taken out of service immediately and marked to prevent its further use.

Article 5

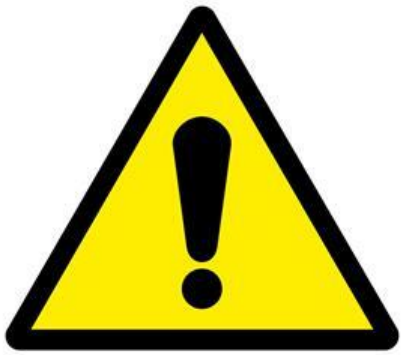
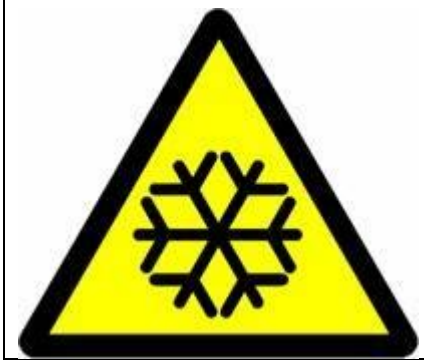
Guidelines for Working, Manipulation and Storing Stainless Steel Pressure Cryogenic Liquids Containers

(1) Only duly trained individuals are allowed to handle cryogenic liquids in pressure vessels. Training is ensured by the head of department.

(2) Prior the first manipulation, if necessary, the manual for stainless steel pressure vessels should be consulted. The manual is kept at the department.

(3) The Operations Department shall keep records of the number and location of stainless-steel pressure cryo-liquid containers. The location/relocation of a cryo-liquid pressure container to/from the laboratory shall be reported by the department head to operations@ceitec.muni.cz . Based on this information, the Operations Department will ensure that the door is marked. Door shall be marked with a combined door sign, see Table 1.

Table 1 – Combined door sign

	**Dusík** **Nitrogen**
	**Kryogenní kapalný dusivý plyn** **Cryogenic Liquid Asphyxiant**

(4) When transporting cryogenic substances, there is a risk of oxygen depletion in the lift. To mitigate this risk, the following steps should be followed:

a) Avoid transport by elevator if possible. Two persons are always required for this activity. One to send the container in the lift, the other to pick it up on the required floor.

b) No persons must travel in the lift with the container!

c) The second person waits on the destination floor and removes the container.

d) The lift can then be released.

(5) It is forbidden to handle pressure cryo-liquid containers on inclined university campus roads (e.g., in underground corridors). Due to the high moment of momentum of the containers, there is a risk of damage to health by running over, pinching persons.

(6) A special trolley should be used to transport large containers of cryogenic liquid outdoors, on pavements and sidewalks. Grates, large cracks, uneven parts, sloping surfaces, other hazards that could catch the wheel and cause tipping should be completely avoided during transport.

Article 6

Guidelines for Handling Cryogenic Liquids

(1) Never touch uninsulated pipes or containers containing cryogenic liquids with any unprotected part of the body. The tissue damage could be the same as frostbite or burns.

(2) Handle Dewar containers with care. Place them only on a flat surface to prevent them from tipping over.

(3) Do not use any tools to scrape the inner glass of the containers. Do not use any hard tools to search the contents inside the containers.

(4) The extremely cold metal will cause the tissue to stick very quickly and tear when trying to separate.

(5) Many substances and materials become brittle on contact with cryogen, and objects made of them can be more easily broken or otherwise damaged – pieces can fly a great distance. Avoid using ordinary glass and plastic.

(6) The release of cryogens into the space can cause damage to the floor, electrical cables and their insulation, pipes, etc.

(7) When overcoming obstacles, the storage vessels may tip over. Handle the containers with utmost care. Ensure that there are no obstacles on the floor. Observe the manufacturer's instructions.

(8) Water from the air can condense and/or freeze in cryogenic liquid storage tanks, leaving water on the floor where workers can slip. The water must be mopped up immediately to avoid a slipping hazard.

(9) If water is constantly condensing in the cryogenic liquid container, the operator must place absorbent materials around the container to prevent the water from becoming a slipping hazard. If the floor cannot be kept dry, it must be clearly marked "Wet floor, slipping hazard!" The marking can be obtained from the cleaning service.

(10) Small Dewar containers with a handle, loose-fitting lid or vent hole can be used for transferring small volumes of cryogenic liquids. This allows the resulting gas to escape at room temperature. If a vacuum flask is used, do not screw on the lid. Personal protective equipment (lab coats, protective shield, cryogenic gloves) must also be used during handling.

(11) Larger volumes of liquid nitrogen should be transported in containers that are mounted either on a stable trolley or on wheels. A minimum of two persons may lift and carry full containers of liquid nitrogen > 25 litres. Dewar containers are heavy and can, for example, crush a leg if run over. Instructions for handling large containers are given in Article 5.

Article 7

Storage Conditions

(1) Store and use cryogens only in places with adequate ventilation (prevention of oxygen-deficient atmosphere, below 19.5%).

(2) The workplace manager is obliged to ensure that cryogens are not stored in confined spaces (risk of suffocation, explosion).

(3) Cryogenic containers are equipped with pressure safety valves, which can be used to control the internal pressure. Under normal conditions, these vessels periodically release some of the gas. It is forbidden to plug, remove or otherwise replace these valves as this may lead to explosion.

(4) Containers should be carried and stored in an upright, bottom-down position.

(5) Small quantities of liquid nitrogen can be stored with care in glass-lined vacuum flask.

Article 8

Working Conditions and Use of Personal Protective Equipment

(1) Work in a well-ventilated area to avoid an oxygen-deficient atmosphere (less than 19.5%). Although the gases are not poisonous or flammable, suffocation could occur in enclosed unventilated spaces. Air that lacks sufficient oxygen content causes stupor, unconsciousness or even death. These gases may not be discernible to the human senses and may be inhaled as normal air. Ensure that adequate ventilation is provided when using these gases.

(2) When carrying and handling cryogenic liquid containers, it is the operator's responsibility to:

a) wear safety shoes, long-sleeved clothing and long but not rolled-up trousers;
b) wear a protective shield or goggles;
c) not to wear contact lenses, metal jewellery, watches.

(3) When decanting liquid nitrogen, wear a lab coat and cryogenic apron.

(4) When handling liquid nitrogen or large cooled objects, wear insulating gloves.

(5) Personal protective equipment must be maintained in a usable condition, damaged equipment must be replaced immediately.

(6) Further information is provided in Annex 1.

Article 9

Authorisation

(1) Only authorized persons with proven knowledge of the operating rules shall have access to the liquid nitrogen laboratory. Access to the laboratory shall be granted by the manager, who shall familiarise the staff with the operating rules.

(2) Only authorized persons over 18 years of age, familiar with all safety instructions, potential hazards and this instruction, may operate liquid nitrogen containers. The responsibility for familiarisation with the above lies with the workplace manager.

(3) Repairs, maintenance, and inspections may only be carried out by adequately qualified persons.

(4) It is prohibited to remove or destroy the labels supplied by the supplier to identify the contents of the container.

(5) All equipment must be kept in perfect order and cleanliness, which is the responsibility of the workplace manager.

Article 10

Hazards while Working with Cryogenic Liquids

(1) When handling liquid nitrogen or helium, any contact with the skin must be avoided due to the risk of frostbite. Splashing on the skin causes burn-like damage. Eyes are extremely vulnerable.

(2) When the human body comes into direct contact with cryogens, the affected parts are rapidly cooled to well below freezing. Freezing is painless, but after thawing is very painful and leads to shock. Also, deeply hypothermic parts of the equipment (e.g., outlet valve bodies, unprotected pipes, etc.) can cause freezing and skin tearing. Even inhalation of cold gases alone can cause serious respiratory damage.

(3) Cryogenic liquids and the cold vapours and gases that emanate from them can have burn-like effects on human skin. Short-term or cursory contact can burn the skin on the face or hands and can damage delicate tissue, for example around the eyes. Prolonged skin exposure or contact with cold surfaces can cause frostbite. The skin then becomes waxy yellow. Initially, the site of contact is painless, but later very intense stabbing pain is reported in the damaged tissues. Unprotected skin may stick to the metal cooled by the cryogenic liquid. When trying to separate the body part from the metal away, the skin may tear and remain on the cold object. Even non-metallic materials are dangerous to the touch and sticky at such low temperatures. Prolonged breathing of very cold air can damage the lungs.

(4) Oxygen is displaced by the escape of helium or nitrogen gas. Oxygen concentrations in the ambient air below 17% to 18% are not sufficient for human respiration. If a helium or nitrogen mist escapes into a room, it is recommended to leave the room and re-enter only when the oxygen content is found to be sufficiently high.

(5) The surface temperature of nitrogen and helium containers can be so low that oxygen or oxygen-enriched air condenses, contributing to fire hazards. If grease, oil, or other flammable material is present in the vicinity of the containers, the escape of cryogenic gases may result in the formation of a potentially flammable liquid due to the liquefaction of air and oxygen concentration.

(6) Accidental leakage (overflow) – can cause damage to property. This leak is most often caused by improper handling. Leakage into a laboratory area poses a risk of suffocation, burns and property damage.

(7) Implosion, the risk can be reduced:

a) Before first use, unpack the container and check for cracks, scratches or chips. Small cracks or scratches in the glass will spread over time and eventually the glass will lose its integrity and break.
b) Before each use, check the container for cracks, dents or scratches.
c) Make sure the container is completely dry before filling, as water trapped in tiny cracks and scratches that subsequently freezes (and expands) can cause breakage.

(8) Careful selection of working materials is necessary. Cold cryogenic liquids can alter the physical properties of many materials, causing them to become brittle and fail.

(9) Always follow the manufacturer's instructions.

Article 11

Laboratories with Sensors Detecting Concentration of Gases

(1) Some of the laboratories have installed sensors detecting oxygen concentration, in Building E35 these are the following rooms: 2S095, 1S152, 241, 239, 1S113, 1S114, 1S115, 1S116, 1S118, 1S119b, 1S119, 1S119c, 1S119d, 1S121, 1S121a, 1S121b, 1S121c, 1S122, 1S124, in Building E26 it is room 1S05, in Building C04 it is room 1S38, 1S41, 1S45 and 1S102.

(2) Detection of reduced oxygen concentrations in the air of selected laboratories, including signalling of the condition, activation of safety extraction and blocking of the use of rooms when the 1st and 2nd level of dangerous concentration is exceeded.

(3) When the oxygen concentration decreases, emergency negative pressure ventilation is triggered.

(4) The laboratory has a traffic light at the door, both outside and inside; the green colour indicates the optimum amount of oxygen in the laboratory.

(5) If the red light is on, there may be a reduced amount of oxygen, leave the lab immediately.

(6) A red traffic light is usually accompanied with audible warning signal.

(7) After leaving the laboratory, contact the manager and the central protection desk (ext. 2929) that will ensure re-measurement of the gas concentration, and the operations department.

(8) The sensors are calibrated regularly once a year. Calibration is provided by University Campus Bohunice Administration ("SUKB", in Czech).

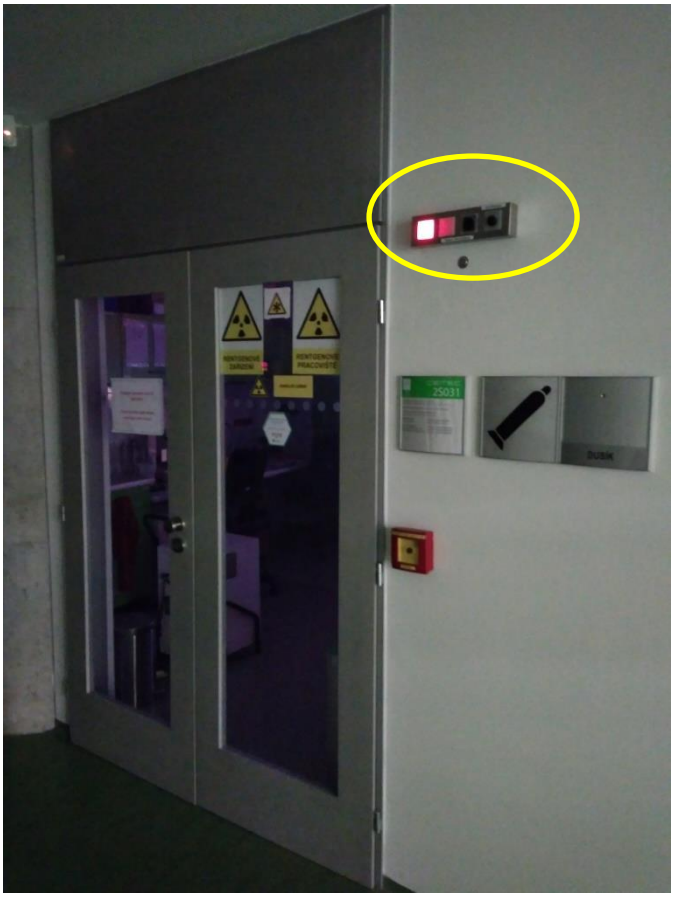


Figure 5 – Oxygen status signalling

Article 12

First Aid Guidelines

(1) Inhalation. In the case of inhalation:

a) Remove or carry the affected person from the infested area to fresh air, bearing in mind also your own safety.
b) Check to see if the affected person is responding.
c) Tilt the affected person's head, lift the chin up and check their breathing.
d) If the affected person is not breathing normally, initiate CPR.
e) Call 155 and follow the operator's instructions.
f) If anyone else is on site, ask to bring a defibrillator (AED). The AED is located in the MRI (Neurosciences) waiting room on the underground floor of Building E35.
g) Place your hands over each other on the centre of the affected person's chest.
h) Compress the chest to the depth of 5 to 6 cm at a rate of 100 to 120 compressions per minute.
i) When using the AED, follow the voice instructions of the device.
j) Upon arrival of the ambulance, continue cardiac massage until paramedics intervene.

(2) In the case of eye contact

a) In the case of eye contact, rinse thoroughly with plenty of water immediately.
b) Arrange medical assistance if necessary.

(3) In the case of frostbite on contact with the skin

a) When frostbite occurs, rinse the affected area with plenty of water.
b) Cover the wound with a sterile drape. Do not remove clothing.
c) Do not rub the frostbitten parts as this may result in tissue damage.
d) As soon as appropriate, place the affected part in a warm water bath, the temperature of which does not exceed 40°C.
e) Arrange medical assistance if necessary.

(4) First aid kits are located in each kitchen in buildings E35 and E26, in the other buildings usually in the corridor behind the partition door.

(5) The injured person is obliged to report each accident to their supervisor and the OSH Manager. The accident must be recorded in the Accident Information Sheet (located in each first aid kit). The accident is recorded by the workplace manager.

(6) The effects of oxygen deficiency can be found in Table 1.

Table 2 – Effects of oxygen deficiency

Volume percentage of oxygen at sea level	**Effects of acute exposure**
> 19.5	None, the atmosphere is not oxygen deficient.
< 19.5	Mild effects similar to exposure at elevated altitude.
< 17	Decreased night vision, increased respiratory volume, increased heart rate, fatigue on exertion.
< 16	Dizziness, accelerated response time, fatigue with mild exertion.
< 15	Impaired attention, impaired judgement, impaired coordination, intermittent breathing, rapid fatigue, loss of muscle control.
< 12	Ver poor judgment, very poor muscle coordination, loss of consciousness, possible permanent damage to the heart or brain.
< 10	Inability to move, nausea, vomiting.
< 6	Spasmodic breathing, spasmodic movements, death within 5-8 minutes.

Article 13

Instructions for Handling Containers

(1) Repairs and maintenance of containers can only be carried out when the container has been depressurised and the contents have been completely evaporated so that the pressure in the container cannot be restored. Repairs and maintenance may only be carried out by personnel who are medically and professionally qualified for the job.

(2) When installing hoses and filler pipes, verify that a safety valve is integrated in each section between the shut-off valves. Liquid gas expands in a confined space when heated and could cause cracking of pipes and hoses and consequent damage to health and equipment.

(3) Use a suitable trolley to move the liquid container.

(4) Transporting cryogenic liquids in an elevator poses a potential suffocation hazard. Transport cryogenic containers without elevator operator. A responsible person in charge will pick up the container on the delivery floor.

(5) Transport cryogenic liquids slowly and at low pressure to minimize evaporation and liquid splashing.

(6) Do not place containers on the ground or roll them around their perimeter. Do not remove or alter fasteners. If the container valve is difficult to handle or the couplings do not disconnect, contact the supplier. Use only the correct and prescribed couplings. Do not use adapters.

(7) Use only original Dewar's closures.

(8) Containers must not be closed tightly! There is a risk of explosion due to pressure!

(9) Store containers only at a well-ventilated place.

(10) Use only spare parts intended for this purpose.

(11) It is always important to read the instructions for use for all containers.

(12) Small quantities of cryogens can be transported between buildings in a suitable low-pressure Dewar container fitted with a lid. Do not carry other items with the container (e.g., books, drinks, samples, instruments, etc.).

(13) Wear appropriate personal protective equipment.
(14) During transportation, it is necessary to ensure that all process valves are closed.

Article 14
Final Provisions

(1) This applies only to workplaces that handle cryogens. Managers of CEITEC MU departments that handle cryogens are obliged to make all their subordinate employees familiar with this measure.

(2) This Measure cancels the Measure of the Director No. 1/2022 Safety when working with liquid nitrogen and other cryogens.

(3) The Head of the Operations Department is responsible for interpreting the individual provisions of this Measure.

(4) The OSH Manager is responsible for the ongoing updating of this Measure.

(5) The heads of individual workplaces are responsible for monitoring compliance with this Measure.

(6) This Measure shall become valid on the execution date hereof.

(7) This Measure shall become effective as of 1. 6. 2023.

Annexes: No. 1 – Personal protective equipment for work with liquid nitrogen and other cryogens

In Brno

Digitally signed by
Mgr. Jiří Nantl LL.M.
17.05.2023

Jiří Nantl
Director
(signed electronically)

Annex no. 1: Personal protective equipment for work with liquid nitrogen and other cryogens

Company:	**CEITEC MU, Kamenice 753/5, 625 00 Brno**
Workplace:	**CEITEC MU, Kamenice 753/5, 625 00 Brno, and other CEITEC MU workplaces**
Profession:	**Specialist (work with cryogens)**
Activity Description: Handling liquid nitrogen and other cryogens. Work with Dewar containers and other equipment for handling cryogens.	

TYPE OF PPE AND LIKELY DURATION OF USE			
Name of PPE	**Details**	**Note**	**Month**
Latex (vinyl, nitrile) gloves	Disposable		
White coat			
Cryogenic gloves			
Protective shield, or goggles, if needed	Clear, chemicals		
Protective apron for working with liquid nitrogen		As required	
Protective footwear		As required	

Table for the selection of personal protective equipment based on risk assessment

Liquid nitrogen and other cryogens			RISKS																									
			PHYSICAL														CHEMICAL (including nanomaterials)					BIOLOGICAL INDICATORS contained in				OTHER RISKS		
			mechanical								thermal		electric		radiation (irradiation)		aerosols		fluids			aero-soles	fluids		materials, persons, animals, etc.			
																	fixed	liquid										
BODY PARTS AND ORGANS TO BE PROTECTED			impact	slip	fall from a height	vibration	static compression of a body part	abrasions, perforations, cuts and other wounds, bites or stings	interception, pinching	noise	heat, fire	chill	electric shock	static electricity	non-ionizing	ionizing	dust, fibres, fumes, vapours	mists, fine mists	immersion	splashing, spraying, splashing out	gases, vapours	solid and liquid	direct and indirect contact	splashing, spraying, splashing out	direct and indirect contact	drowning	lack of oxygen	lack of visibility
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
head	skull	A																										
	whole head	B																										
ears / hearing		C																										
eyes / eyesight		D																										
face		E																										
respiratory organs		F																										
hands		G										X						X	X	X								
arms (parts)		H																										
legs (feet)		I																										
legs (parts)		J																X		X								
skin		K										X						X		X								
body / abdomen		L																										
body part		M																										
whole body		N										X						X		X								