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Guidelines for sampling, transport, storage and chemical characterisation of environmental and human samples

February 2009, V 2.0.0

1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for the monitoring of the environment. It is in the responsibility of the Federal Ministry for the Environment, Nature Protection and Reactor Safety (BMU) and technically and administratively coordinated by the Federal Environment Agency (Umweltbundesamt). The ESB collects ecologically representative environmental specimens as well as human samples, stores them and examines the archived material for environmental relevant substances.

The long-term storage is performed under conditions that exclude a change of state or a loss of chemical characteristics as far as possible during a period of several decades. By this means the archive provides specimens for a retrospective monitoring of such substances, whose hazard potential for the environment or human health are not yet known.

Comprehensive information on the German ESB is available at www.umweltprobenbank.de (English language pages available).

2 General information

This protocol applies to biological environmental samples like bladder wrack, bream muscle, earthworm, spruce shoots as well as abiotic samples such as soil and suspended particulate matter.

For the storage in the ESB archive all samples are frozen immediately at the time of the sampling and the cooling chain is not interrupted at any time afterwards. Thus transport, storage as well as grinding and homogenization of the sample material takes place under cryogenic conditions (temperature below approx. -130°C) considering special safety regulations.

The particularly high quality assurance requirements result from the extraordinary importance of the samples as archive material. Representativeness and reproducibility of the specimens are prerequisites for the comparability of the analytical data in time and space.

The aim of this guideline is to provide information on the safe transport of sensitive sample material under cryogenic conditions in compliance with safety regulations and without deterioration of sample quality.

3 Terminology

3.1 Cryogenic conditions

Cryogenic conditions exist at temperatures of below approx. -130°C (approx. $< 140\text{ K}$). The temperature is therefore below the glass transition temperature of water. No further re-crystallisation of ice takes place and no further ice crystals form (BURDEN 1999). This ensures that chemical processes in the samples are reduced to a minimum and morphological changes, e.g. through further growth of ice crystals, are prevented. During storage in the gas phase over liquid nitrogen, an inert-gas atmosphere is created by the nitrogen evaporating in the sample-storage container. This also prevents any changes in the samples caused by oxygen from the atmosphere (oxidation processes).

3.2 Transfer report

A document certifying the transfer of samples and containing at least the following data:

- Transferring organisation (address, contact person, telephone no. and e-mail address),
- Recipient of samples (address, contact person, telephone no. and e-mail address),
- List of samples and description including ESB codes,
- Date and place of transfer,
- Signature of recipient and representative of transferring organisation.

4 Transport process

4.1 Safety notes for working with liquid nitrogen

Caution: Liquid nitrogen is extremely cold (i.e. skin and tissue may be damaged on contact) and may

cause asphyxiation when evaporating. When liquid nitrogen is being used in enclosed rooms, sufficient ventilation is essential. The oxygen content of the room atmosphere should be monitored with a portable oxygen sensor or a stationary oxygen-deficiency alarm system (settings: e.g. first alarm at oxygen contents ≤ 19 %, and main alarm at ≤ 17 %).

4.2 Preparations

The transport container(s) (vacuum-insulated stainless-steel containers with lid of insulating material) should be cooled down to the required temperature at least two days before the transport operation.

The samples are stored on a platform in the previously cooled transport container in such a way that they do not come into direct contact with the liquid nitrogen (in suitable containers or racks which are secured to prevent them toppling over).

The sample vessel should not be closed tight since any liquid nitrogen entering it may cause the vessel to explode when it is warmed up.

Before beginning the transport operation, staff members should receive instruction on the regulations regarding the transport of hazardous goods on public roads.

4.3 Material and apparatus

For the transport in vehicles on public roads, the regulations governing hazardous substances apply (especially: labelling of the cryogenic containers, availability of fire extinguisher and torch light). The issuing of transport documents is not required if the transport is taking place within Germany for the user's own purpose and the quantity of liquid nitrogen is less than 1000 kg.

The following equipment is required for transport under cryogenic conditions.

4.3.1 An insulated transport container suitable for cooling with liquid nitrogen and with suitable storage racks for the samples. Labelling: UN 1977 nitrogen, deep frozen, liquid. Hazard label no. 2.2 (gas cylinder against green background).

4.3.2 Liquid nitrogen for cooling. For transport over longer periods also in a separate storage container. (If this container is being transported in the closed state, it must be approved as a pressure vessel for road transport: type labelling TT). Hazardous-material labelling: UN 1977 nitrogen, deep frozen, liquid. Hazard sheet no. 2.2 (gas cylinder against green background).

4.3.3 Plunger for measuring the level of liquid nitrogen.

4.3.4 Suitable thermometer or thermocouple for measuring temperature.

4.3.5 Torch light with no metallic surface.

4.3.6 Fire extinguisher (2 kg, powder extinguisher; fire classes A, B, C; complying with standard EN 3; with valid test seal).

For the transport of ESB samples, the following equipment is used:

- Cryogenic-transport container, volume 400L manufactured by Cryotherm GmbH, Euteneuen, or cryogenic-transport container, volume 100 L, type CS100B, manufactured by Cryo Anlagenbau GmbH, Wilnsdorf.
- Transportable liquid-nitrogen storage container, volume 210 L, type ABC-200-VTB (π), manufactured by Achenbach Cryotechnik, Kreuztal (approved for road transport according to hazardous-goods regulations).
- Transportable liquid-nitrogen storage container, volume 50 L, type Apollo 50, manufactured by Cryotherm, Euteneuen (for unpressurised transport only).

Sample vessels of the following types are used:

- Glass vials (e.g. 20 mL scintillation vials purchased from PerkinElmer, Rodgau-Jügesheim) of high-quality glass with following standard dimensions: height approx. 60 mm, diameter approx. 25 mm. The screw caps are of plastic with metallic foil on the inside.
- Glass flasks, volume 100 mL, of borosilicate glass with screw-on plastic caps.

- Stainless steel containers (Bain-Marie inserts according to Gastronorm) for the transport of non-homogenized raw samples; tight but not gas-proof closed with stainless steel covers and stainless steel cramps, sizes: 1.5 L, 3.5 L und 5 L.

4.4 Cleaning instructions

Following transport, the cryogenic transport containers should be allowed to warm up to room temperature. The surface should then be washed with a mild cleaning agent and allowed to dry completely. The cover should not be replaced until the container is dry. The cleaning procedure is important in order to avoid health hazard through microbe contamination.

4.5 Storage at destination

At their destination, the samples should be stored in a suitably pre-cooled cryogenic-storage container.

5 Documentation

For every transport of samples, an accompanying certificate as well as a transfer report should be completed. The exact form and content of these documents varies from case to case (an example is shown in Appendix A).

6 References

BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Hrsg.) (2008): German Environmental Specimen Bank – Concept (Status: October 2008); www.umweltprobenbank.de

Burden, D. W. (1999): Issues in Contamination and Temperature Variation in the Cryopreservation of Animal Cells and Tissues. Revco Technologies, Asheville, NC 28806 (USA), Application Note 99-08

Umweltbundesamt (1996): Umweltprobenbank des Bundes - Verfahrensrichtlinien. Herausgeber: Umweltbundesamt, Berlin. Erich Schmidt Verlag, Berlin.

Appendix A: Example of transfer report

German Environmental Specimen Bank - Document for Transport and Transfer of Samples -

Transferred by: Federal Environmental Specimen Bank
c/o Fraunhofer Institute for Molecular Biology and Applied Ecology
Auf den Aberg 1
D-57392 Schmallenberg
Tel. +49 (0) 2972 3020, Fax +49 (0) 2972 302319

Contact: NN

Delivery to: NN

Contact: NN

Samples in ESB standard vials (scintillation vials):

Type of sample / no. of vials	Sampling area / Year	ESB code / remarks
Eelpout musde 6 STP	Varel-Mellum 2001	4210/0/0001/07303/0/
Eelpout musde 4 STP	Meldorfer Bucht 2001	4210/0/0601/07202/0/
Eelpout musde 6 STP	Darßer Ort 2001	4210/0/0601/06103/0/
Eelpout musde 6 STP	Varel-Mellum 2002	4210/0/0502/07303/0/
Eelpout musde 6 STP	Meldorfer Bucht 2002	4210/0/0502/07202/0/
Eelpout musde 6 STP	Darßer Ort 2002	4210/0/0602/06103/0/
Herring gull egg 6 STP	Trischen 2001	4311/0/0501/07201/0/
Herring gull egg 6 STP	Mellum 2001	4311/0/0501/07301/0/
Herring gull egg 6 STP	Heuwiese 2001	4311/0/0401/06201/0/
Herring gull egg 6 STP	Trischen 2002	4311/0/0602/07201/0/
Herring gull egg 6 STP	Mellum 2002	4311/0/0502/07301/0/
Herring gull egg 6 STP	Heuwiese 2002	4311/0/0402/06201/0/
Domestic pigeon eggs 6 STP	Halle 1997	1211/0/0097/11101/0/
Domestic pigeon eggs 6 STP	Saartal 1999	1211/0/0099/02100/0/
Domestic pigeon eggs 6 STP	Bornhöved / Belauer See 1999	1211/0/0099/03100/0/

Record of transfer:

Place and date of transfer:

Transferred by (name, signature)

Received by (name, signature):

Copy of recipient / sender (delete as applicable)

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Appendix B: Instructions for working with liquid nitrogen

DANGER FOR PERSONS AND ENVIRONMENT

On contact with eyes or skin, cryogenic, liquid nitrogen causes serious frostbite with injuries similar to burns (i.e. inflammation, swelling, blistering) as well as severe damage to tissue. The cold gas is heavier than air, accumulates at floor level and may displace the oxygen in low-lying rooms. One litre of liquid nitrogen produces approximately 650 litres of gas! At concentrations of more than 85% in the air, severe oxygen deficiency occurs causing symptoms such as drowsiness, nausea, increase in blood pressure and difficulty in breathing. Concentrations of 88% and more, lead to immediate loss of consciousness and risk of asphyxiation.

SAFETY MEASURES AND PROCEDURES

Liquid nitrogen should be transported and handled only in suitable cryogenic vessels or apparatus which is resistant to cold. Never handle larger quantities in small poorly ventilated rooms. Always avoid contact between the cryogenic liquid or gas and the skin and eyes. Always wear protective clothing, impermeable shoes, leather safety gloves and goggles. If release of large quantities of gas is anticipated or unavoidable, also use insulating equipment. When working in confined or inadequately ventilated rooms, a second person is required for supervision outside the danger zone who can raise the alarm if necessary.

EMERGENCY PROCEDURE

Fire: Nitrogen is not inflammable. Take measures appropriate to the surroundings. If a fire cannot be extinguished immediately, leave the area immediately. Never attempt to extinguish flames with liquid nitrogen. On release of larger quantities of nitrogen, warn all other persons, leave the danger zone and re-enter it only with insulating equipment. If possible, repair the leakage. Do not re-enter the danger zone without isolating equipment and before it has been thoroughly ventilated. If necessary, measure the concentration of nitrogen in the air.

FIRST AID

Aspiration: Take affected person out of doors immediately. If necessary assist respiration with mask and bag to provide ventilation. Seek medical assistance! Eye contact: do not move or rub parts of the body which are frozen rigid. Thaw carefully with (cold) water. Remove clothing and cover the body loosely with sterile bandaging material. Seek medical assistance!

DISPOSAL

Allow to evaporate slowly in the open air. Loosely cover the opening of the vessel to avoid condensation and concentration of atmospheric oxygen in the remaining liquid.

Appendix C: Transport of Samples Cooled with Liquid Nitrogen

Status: February 2007

Up to a net maximum of 1000 kg liquid nitrogen, transport may be carried out in a standard vehicle (up to a maximum weight of 3,5 t. for vehicle and trailer, if in use). The density of liquid nitrogen is 812 kg/m³ (for conversion to volume).

The load must be secured to prevent it from moving. The transport compartment of the vehicle should be ventilated (e.g. ceiling ventilator). If this is not the case, the vehicle ventilation system should be switched on and the windows opened if necessary.

The following regulations apply for the transport container (ADR):

- The material must not be corroded by liquid nitrogen.
- The transport container must be able to withstand the normal stresses of transport.
- The quality of the material selected should ensure that the closing device does not work loose from the container.
- The transport container must be approved for the transport of liquid nitrogen.
- The transport container must withstand the pressure (if valve present).
- Double-walled containers must be used which prevent liquid from shooting out under pressure, e.g. vessels with vacuum double walls.
- The transport container must be approved for transporting hazardous substances.

The outer packaging should be marked as follows:

UN 1977 nitrogen, deep frozen, liquid.

Hazard label no. 2.2 (gas cylinder on green background) must be applied to the container. If the direction of the interior compartment is not apparent, this should be indicated by the double arrow symbol on the outer packaging.

Other equipment:

2 kg fire extinguisher (class A, B, C, according to standard EN 3 with valid test seal; test interval, 2 years).

Torch light with non-metallic surface.

Emergency procedure sheet (not compulsory).

Transport certificate:

Transport certificate is not necessary if the goods are being transported within Germany for internal purposes, i.e. not by shipper, courier service etc.

If a transport certificate is required, it must contain the following information:

Sender (name and address);

Recipient (name and address);

"UN 1977 nitrogen, deep frozen, liquid, 2.2".

The type of packaging (e.g. cryogenic container) and the quantity must be stated and marked as follows:

"Transport within restrictions stated in sub-section 1.1.3.6".

Appendix D: Emergency-procedure sheet for transport of liquid nitrogen on public roads

LOAD IDENTIFICATION

Class 2, Cl. Code 3A, 22, UN 1977

CHARACTERISTICS OF LOAD

Colourless, odourless, deep-frozen liquified gas.

DESCRIPTION OF HAZARD

Leaking fluid is extremely cold and evaporates rapidly. Liquid causes frostbite and serious injury to the eyes. The gas may cause asphyxiation without apparent warning. The gas is invisible, heavier than air and accumulates on the ground. It forms visible vapour when in contact with moist air. Contact with heat leads to an increase in pressure which may cause closed containers to explode. Does not generally pollute water (WGK 0).

PERSONAL SAFETY EQUIPMENT

Signal jacket, goggles, suitable plastic or leather gloves, strong shoes, hand lamp.

GENERAL ACTION TO BE TAKEN BY DRIVER IN EMERGENCY

Switch off the engine. No naked flames. No smoking. Set up warning sign on road. Warn other road users and passers-by. Provide information on type of hazard. Remain upwind of hazard. Summon police and fire brigade.

SPECIAL ACTION TO BE TAKEN BY DRIVER IN EMERGENCY

If possible remove leakage. Allow fluid to evaporate. Prevent fluid from entering drainage system or low-lying rooms. Gas causes asphyxiation. Ensure own safety.

FIRE; INFORMATION FOR DRIVER IN CASE OF FIRE

Extinguish source of fire only. Liquid nitrogen itself is not inflammable.

FIRST AID

Warm parts of body which have contacted the fluid with water. Remove clothing carefully. Seek medical assistance in case of symptoms due to aspiration or contact with skin or eyes.

ADDITIONAL INSTRUCTIONS

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APPLIES ONLY FOR ROAD TRAFFIC!