

Appendix 1: Laboratory Rules for Work Activities and Handling Laboratory Equipment

Director's Directive 3/2019 - RECETOX Operating Regulations

Preliminary Provisions

The rules described in this Appendix to the RECETOX Operating Regulations apply to all RECETOX laboratories.

Basic Rules

1. All work performed in the laboratories must be compliant with the Laboratory Operating Procedures, the OHS fact sheet and good laboratory practice.
2. Staff are required to use personal protective equipment when working in the laboratories.
3. Only persons trained to handle pressure cylinders are permitted to handle and work with pressure cylinders and technical gas distribution systems.

Working with Chemicals

1. Laboratory staff are required to acquaint themselves with the material safety data sheets (MSDS) – and, if required, further information in toxicology databases – concerning all the chemicals they use, or will use, at work. They must heed all the safety information listed for the particular substance.
2. When working with chemicals, staff must heed the warning symbols and follow all the instructions stated on the chemical label and in the material safety data sheet, particularly all the instructions on minimising any hazards to staff health and safety. Staff must also be familiar with the signs and symptoms of exposure and seek medical help as soon as they

become aware of any signs of poisoning. Staff must know the special procedures for each substance used in case of any chemical accidents.

3. Prior to handling any substances that are potentially hazardous to human health, all technical and organisational measures taken for health protection must be carefully checked and decontamination agents made ready for any accidents.
4. Toxic substances (marked with a T and the skull-and-crossbones symbol), highly toxic substances (marked with T+ and the skull-and-crossbones symbol) and substances assigned the hazard statement codes H340, H350, H350i, H360F or H360D must not be stored in the laboratory. When not in use, these substances must be stored in the toxic chemicals store. Only staff members that meet the statutory requirements under **section 44(b) of Act no. 258/2000 Coll.** may work with these substances. All other staff members must provably complete one-on-one training provided by a person authorised to do so under **section 44(b) of Act no. 258/2000 Coll.** before working with these chemicals. The training is provided by the OHS and FS Officer. A record of this training is entered in the personal information sheet of the staff member and kept in the archive maintained by the OHS and FS Officer.
5. (Highly) hazardous (biological) agents and toxins ((H)HAT) as defined by **Act no. 281/2002 Coll.** and the relevant implementing decree (see <http://www.rect.muni.cz/nso/>) are subject to special regulations. Compliance with these regulations is supervised by the RECETOX Specialised Representative for (H)HAT. Only persons trained by the Specialised Representative may handle the (H)HAT. The Specialised Representative must be informed in writing of the acquisition, possession, use, development, production, import, export, transport or consumption of (H)HAT without undue delay (within two working days). The Specialised Representative monitors the management of (H)HAT within the intention of Act no. 281/2002 Coll., approves (H)HAT purchase orders and maintains the inventory records of (H)HAT on the RECETOX premises. The Specialised Representative prepares the annual statement of (H)HAT management submitted to the State Office for Nuclear Safety.
6. Laboratory staff must request that their direct superior provides them with all the equipment etc. required for the protection of health (including special protective equipment, workplace and special laboratory equipment) according to the symbols and the instructions on the labels and in the material safety data sheets for the chemicals they work with. Until these measures have been taken, laboratory staff members must not work with the chemicals in question and must assert their right to refuse to work with these chemicals.
7. The following rules apply when working with toxic substances, potentially pathogenic cultures and cell cultures:
 - Single-use instruments (such as pipette tips) are placed in a disinfectant solution or in a designated container after use.
 - Live cultures are sterilised before they are disposed of; they are not to be poured straight into the drain.
 - In the case that any work surface, floor or laboratory equipment is soiled with live cultures, the following procedure applies: the incident is reported to the head of the project and the General Laboratory Manager and/or Technical Laboratory Manager and all soiled surfaces are thoroughly washed with water and sterilised with a 70% solution

of ethanol. If the soiled object is an item of clothing, it must be immediately removed, placed in a polyethene bag and immediately sterilised, rinsed under running water and washed. If live cultures are spilt on skin or mucous tissue, the affected area must be washed under running water for at least 10 minutes and, if applicable, neutralised.

8. When working with potentially pathogenic microorganisms, laboratory staff must wear coats and gloves and work in the “hazard box”.
9. When working with pulverulent materials, laboratory staff must use a respirator and work under a dust hood.
10. Solid chemicals must not be touched with unprotected hands.
11. All liquids – particularly corrosive, toxic and infectious liquids – may only be pipetted using micropipettes or glass pipettes with controllers. It is forbidden to pipette any liquids by mouth.
12. When handling substances in test tubes and open vessels, the opening must always point away from the person handling the substance and other nearby staff.
13. Containers with toxic substances or corrosives must not be moved when open. Solid toxic substances must be transferred from their containers with instruments made from materials that do not react with the particular substance.
14. Corrosives must not be stored higher than the shoulder level of the staff member who handles them and no higher than at 165 cm.
15. Solid hydroxide must be dissolved by gradually adding small amounts to water while constantly stirring. Water must never be poured onto hydroxide.
16. When handling concentrated acids and lyes, staff must protect their eyes with a visor or glasses and their body with a rubber apron. Concentrated acids must only be handled in a fume hood. Vessels containing acids must be placed on a dish.
17. When diluting an acid, always pour the acid into water and never the other way around. Acids must be poured slowly and carefully, particularly sulfuric acid.
18. Nitric acid that is spilt must not be removed using sawdust, cloths and other organic matters. It must be neutralised before removal or, if that is not practicable, diluted as much as possible. Glassware soiled with organic matter must not be cleaned using nitric acid, as that could result in violent reactions, creation of nitric oxides and spontaneous combustion. Cleaning nitric acid spills must be done so as to avoid contaminating a large area.
19. Any spilt acids, particularly concentrated acids, must first be carefully diluted with water and slightly neutralised by sprinkling the spill with carbonates (such as soda or chalk) or pouring a diluted alkaline solution over the spill. The spill is then carefully washed away with water or allowed to soak into sawdust, cloths etc.
20. Spilt perchloric acid must first be diluted with a large amount of water and then removed using non-flammable material. Never use cotton or cellulose. The material used to remove perchloric acid must be washed immediately under running water.

21. Cleaning supplies are located in a designated place in each laboratory or set of laboratories and are included in the list of available equipment in the laboratory OHS fact sheet.
22. Bottle stoppers must be placed so that the soiled surface does not touch the work surface to reduce the risk of chemical burns, poisoning and contamination.

Working with Flammables

1. A maximum of 20 l of liquids with a low boiling point (such as ether or carbon disulphide) in unbreakable containers or a maximum of 10 l in one-litre breakable containers may be stored in laboratories and stores located in the same fire compartment, provided they are stored separately from other flammables. (ČSN 65 0201)
2. The amount of other flammables stored in a single fire compartment must not exceed 250 l, including no more than 50 l of category I flammables (such as acetone, benzene, petrol, butanol, cyclohexane, cyclohexanol, ethanol, ethyl acetate, hexane, isopropanol, methanol, methyl acetate, pyridine and nitrobenzene). If these flammables are stored in breakable containers, the containers must not be larger than 5 l and must be stored in a closable and correctly labelled fire-resistant cabinet.
3. Flammables must be used in such a way as to prevent spillage beyond the work surface. The laboratory must be sufficiently ventilated. The temperature in the room where these substances are stored must not exceed 35°C.
4. Laboratory staff must only handle organic solvents, fuming substances, malodorous substances, substances that release toxic or irritating gases and flammable substances (classified as F or F+) in a switched-on fume hood or while wearing a respirator.
5. It is forbidden to use a naked flame around solvents. Flammable liquids must only be handled in the fume hood.
6. When heating flammable liquids, it is necessary to prevent superheating by adding boiling chips or glass tubes.
7. When heating liquids in heated baths, the liquid used for the bath must be miscible with the heated liquid. Water baths may be used when evaporating small amounts of liquids with low boiling point and when using tabletop rotary evaporators.
8. It is forbidden to leave the apparatus unsupervised when distilling flammable liquids or using the extractor. When water cooling is used, the supply of water to the cooler must be monitored.
9. Separation processes involving flammable liquids require measures to prevent explosive mixtures from forming in the atmosphere in the laboratory.
10. In the case of spilt flammable liquid, staff must immediately switch off any gas appliances, shut off the power supply outside the room, impose a no-entry restriction for unauthorised

persons and ensure proper ventilation by opening the windows. The spilt flammable liquid is allowed to soak into a suitable porous material, which is then placed in a metal container with a lid and disposed of in accordance with Act no. 185/2001.

11. Spilt non-polar solvents must not be wiped on the floor or a plastic surface (danger of static discharge).

Working with Biological Material

Biological material is material of environmental and biological origin that comes from humans, other living organisms or parts of the environment. The rules for working with biological material are described in detail in Appendix 2 to these Operating Regulations (“Laboratory Rules for Working with Biological Material”). Biological material processed in RECETOX laboratories is treated as potentially infectious material. Working with biological material is only permitted in select laboratories adapted for that purpose and governed by separate rules. Only trained staff can work with biological material. Furthermore, only persons vaccinated against hepatitis B are authorised to work with biological material. Any reusable supplies used when working with biological material must be regularly decontaminated and sterilised. The disposal of waste created while working with biological material is governed by separate rules.

Labelling Samples and Solutions

1. All prepared solutions, samples and materials (in the laboratories, refrigerators, incubators, incubator rooms etc.) must be labelled and kept separated from the material used by other staff members (in boxes, separate racks etc.). Unlabelled samples will be removed without notice.
2. The label on each solution, sample etc. **must follow the pattern below:**
 - Name;
 - Volume and concentration;
 - Date of preparation (the full date including the year);
 - Name of the staff member (e.g. John Smith or initials – JS).

Chemical Management

RECETOX Specialised Representative for (H)HAT

The RECETOX Specialised Representative for (H)HAT manages, stores and issues chemicals classified as HAT or HHAT and maintains the inventory records of these substances. These substances are only issued in the amounts required for the experiment.

OHS and FS Officer

The OHS and FS Officer manages chemicals classified as T or T+ including storage, inventory records and distribution.

These substances are only issued in the amounts required for the experiment.

RECETOX chemical management rules

1. The chemical inventory records and their distribution are managed by the Technical Laboratory Manager together with the General Laboratory Managers of individual laboratories.
2. The inventory records of the chemicals are kept in the central database of chemicals and the chemicals are stored in the following locations:
 - **Central store for chemicals and solvents:** rooms 1S42, 1S43 and 1S44.
 - **Store for hazardous chemicals:** chemicals classified as T, T+ and assigned the risk phrases R45, R46, R49, R60 and/or R61 require keeping strict inventory records in accordance with the guidelines for handling hazardous chemical substances, preparations and objects at Masaryk University (*Pokyny pro zacházení s nebezpečnými chemickými látkami, přípravky a předměty na Masarykově univerzitě*, <http://www.rect.muni.cz/nso/>) and are stored separately in a locked chemical cabinet and/or a locked refrigerator. The chemicals are distributed by the OHS and FS Officer or their deputy who meets the requirements under section 44(b) of Act no. 258/2000 Coll. The distribution must be recorded in the inventory records for hazardous chemical substances, preparations and objects (*Evidenční kniha nebezpečných chemických látek, přípravků a předmětů*).
 - On-site storage: small amounts of chemicals required for current or regular experiments and chemicals that are only used in one laboratory can be stored on-site at the laboratory in a designated laboratory cabinet and/or refrigerator.
 - **Store for highly hazardous agents and toxins (H)HAT:** the store is located in room 1S23. Only persons trained by the Specialised Representative for (H)HAT have access to the store.
3. Chemicals taken from the store or another designated place must be returned to their original location immediately after completing the task. Chemicals are added to the inventory records when delivered and the Technical Laboratory Manager records the month and calendar year of delivery on each chemical as soon as they are delivered. The chemicals are also added to the list of chemicals (electronic database).
4. The chemicals are numbered with a permanent marker so that they are easily recognised in the store. The material safety data sheets (MSDS) concerning the chemicals that are currently used are kept in a separate folder at a clearly marked location in the laboratory.
5. Chemical substances classified as T or T+ and assigned the hazard statement codes H340, H350, H350i, H360F and/or H360D are subject to special regulations, as described above.

Device Operating Rules

Prior to using a device or an item of laboratory equipment, each staff member must be provably trained in operating the device (training and record in the device log). This training is provided by the Device/Equipment Manager.

1. The staff must enter a complete record into the device log after each use.
2. Devices must be used in accordance with the manual provided by the manufacturer.
3. Any device malfunction must be reported to the Device Manager and/or the staff member of the Operations Department responsible for building management.

Device log

The users and Device/Equipment Managers are responsible for the records kept in the device log.

The device log includes:

- Name and type of device or equipment, inventory number and commissioning date;
- Name of the staff member responsible for the device;
- Names of the staff authorised to use the device (users);
- Records of user training;
- Records of device use (date, name, purpose, signature);
- Records of device malfunctions and corresponding repairs.

Rules for Handling Pressure Cylinders and Liquified Gas Containers

General requirements, signs at the workplace

1. Metal pressure cylinders and barrels containing compressed or liquified gasses may only be placed in the workplace based on prior agreement with the faculty expert on fire safety.
2. Containers that are currently being used and spare containers must not be placed in locations where they could represent a safety hazard, such as cellars, pedestrian and vehicle passages, escape routes and staircases, offices, changing rooms, kitchens, unventilated areas, areas that are difficult to access and publicly accessible areas. Pressure cylinders must not be placed in basements or below ground level (section 44(6) of Decree no. 246/2001 Coll.). Pressure cylinders must only be placed in designated locations.
3. The number of cylinders (calculated for cylinders with an internal volume of 50 l) in a single room where these cylinders are used, regardless of whether they contain the same gas or different gasses, must not exceed 12. When a fire compartment includes more rooms where pressure cylinders are used, the total number of cylinders within the fire compartment must not exceed 24 (calculated for cylinders with an internal volume of 50 l). The placement of cylinders in laboratories is subject to ČSN 01 8003.
4. The laboratories are not designed for storing pressure cylinders. Cylinders that are not being used (such as empty or spare cylinders) must be placed in the designated locations in the corridors within the laboratory areas.

5. Safety signs are used to mark the locations designated for placing currently unused pressure cylinders and barrels. Notices containing information about the presence and number of pressure cylinders are located at the entrance to the building.

Safe use of pressure cylinders

1. Only RECETOX staff who are aged 18 years or older, medically fit and authorised for the task and who have provably completed their training, and suppliers may handle, transport and store pressure cylinders and handle compressed gas barrels.
2. Staff who empty or otherwise handle the cylinders (storage, transportation etc.) must be provably instructed in the user manual once every three years before they can be authorised to fulfil this task. The training is provided by external experts who are qualified to provide such training.
3. RECETOX staff who have completed the training for handling pressure cylinders and/or tanks containing liquified gas are listed on the RECETOX [INTRANET](#).
4. It is forbidden to use a naked flame in a laboratory where a pressure cylinder containing nitrogen is located.
5. Containers with toxic and corrosive gases must only be filled and handled in the presence of at least two persons. These persons must be qualified and medically fit for the task. All workplaces where containers with toxic and corrosive gasses are handled must be permanently equipped with no fewer than two self-contained breathing apparatuses.

Handling gas containers

1. Each gas cylinder must be secured with a safety chain at two-thirds of its height to prevent it from falling over or moving around.
2. It is forbidden to store any flammable substances within 5 metres of the cylinders or work with a naked flame without permission.
3. The containers and barrels must be placed at a sufficient distance from heaters and surfaces radiating heat so that the surface temperature of the containers does not exceed the critical temperature for liquified gasses and 50°C for other gasses.
4. The containers/cylinders and barrels must be placed at least 3 metres away from any sources of a naked flame.
5. Containers not currently in use and provided with a removable cap must have the cap in place.
6. The cylinder valves must not be opened and closed using pliers or adjustable wrenches. The correct recommended wrench must be used instead. The valves must be opened and closed slowly.
7. The cylinder pressure regulators must be opened and closed manually without any tools or instruments and in a slow and steady manner.

8. It is forbidden to oil cylinder valves! The cylinder valves must not be connected to nuts with damaged threads or nuts with other types of threads designed for other gasses.

A general guide for commencing work with a pressure cylinder:

- Close the pressure regulator drain valve.
- Connect an overpressure-sensitive device.
- Open the main cylinder valve.
- Set the output pressure on the pressure regulator.
- Slowly open the pressure regulator drain valve.
- If necessary, adjust the output pressure.

A general guide for finishing work with a pressure cylinder:

- Close the pressure regulator drain valve.
- Disconnect the overpressure-sensitive device/put away samples.
- Close the main cylinder valve.
- Carefully let out the excess pressure from the pressure regulator by opening the pressure regulator drain valve and leave it open.

Laboratory Waste Disposal

1. All waste is disposed of in accordance with the Operating Regulations of the University Campus Bohunice – Appendix no. 6 (*Provozní řád odpadového hospodářství UKB*). Disposal of waste produced at the laboratories is managed and monitored by the Technical Laboratory Managers.
2. In accordance with the UKB waste disposal rules, the waste produced at RECETOX is divided into the following categories:
 - Hazardous (*nebezpečné* – N): everything that has come into contact with a toxic substance or that is toxic, infectious or otherwise dangerous;
 - Other (*ostatní* – O): mixed municipal waste;
 - Secondary raw materials – uncontaminated, to be recycled.
3. Further details about the RECETOX waste in each category, waste collection and storage and removal by the Management of the University Campus Bohunice can be found on the [INTRANET](#).
4. Mixed municipal waste – uncontaminated, is placed into the waste bins in the laboratories and offices. Substances representing a fire hazard must not be placed into the waste bins.
5. Secondary raw materials – uncontaminated, to be recycled, are placed into the boxes and bins designed for recyclable waste located in the interim waste storage area on the 1st basement floor by building A36 (municipal waste, glass, plastic, paper, cardboard boxes, polystyrene, toner cartridges, electrical waste, batteries, fluorescent lights, metal waste, wood).

6. Recyclable uncontaminated waste may also be placed in the bins in the corridors on the 2nd and 3rd floor in front of the entrances to building A29.
7. Each laboratory has closable bins or other containers that can be easily transported by the staff of the University Campus Bohunice waste management centre for storing hazardous waste (N) produced in the laboratory. The bins and containers are labelled in accordance with Act no. 185/2001 Coll. and Decree 381/2001 Coll. of the Ministry of the Environment of the Czech Republic.
8. The labelled bins and containers containing hazardous waste (N) are stored in labelled metal cabinets in the laboratory that produced the waste or in a designated waste collection location, ready for regular collection and disposal by the staff of the University Campus Bohunice waste management centre. Records are kept of the amount and type of waste disposed of.
9. The laboratory bench drains leading to a tank collecting chemical wastewater can only be used for disposing of the remaining quantities of chemical solutions miscible with water in amounts that do not represent a hazard for the water environment and for water-soluble solvents (up to 0.5 l, diluted at least ten times), acids and hydroxides (diluted 30 times with pH around 6.5–8.5) if such waste cannot be collected in waste bins for operational reasons.
10. It is forbidden to use any drain to dispose of solvents immiscible with water, toxic and highly toxic substances, flammables and explosives, concentrated acids and hydroxides and compounds that release toxic or irritating substances upon contact with water, acids or bases.
11. Used and waste solvents (neutralised and without pyrophoric substances) are collected in labelled glass containers according to the waste classification and stored in the designated laboratory cabinets.
12. Used chromosulfuric acid is placed in a separate bottle that must only contain this acid.
13. Chemical glassware that has been used for toxic or corrosive substances must be thoroughly rinsed out and washed before further use. The solutions resulting from these activities are stored in the designated waste bins for hazardous waste or poured down the drains that lead to the tank collecting chemical wastewater located under building A29 (see above).
14. The gloves used when working with toxic or corrosive substances are collected in the designated closable hazardous waste bins.
15. Loose non-hazardous materials (such as chromatographic stationary phases) must be wrapped in a piece of paper or placed in a bag before they are placed in a waste bin.
16. Please use the following form to submit waste collection requests:
<https://odpady.ukb.muni.cz/>.

Final Provisions

1. The person authorised to interpret the provisions of this Appendix and ensure that they are complied with is the OHS and FS Officer.
2. The person authorised to interpret the provisions concerning (H)HAT and ensure that they are complied with is the Specialised Representative for (H)HAT.
3. This Appendix to the RECETOX Operating Regulations becomes effective on the date of signature by the RECETOX Director.

Brno, 9 September 2019

.....
prof. RNDr. Jana Klánová, Ph.D.
Director of RECETOX