U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

LABORATORY SAFETY MANUAL

MANUAL PROPOSED BY THE COORDINATING COMMITTEE ON LABORATORY SAFETY

APPROVED BY THE DIRECTOR ON APRIL 30, 2024

THE DIRECTOR OF FCUP

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(FULL PROFESSOR)

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1. GENERAL ORGANIZATIONAL ASPECTS

1.1 FRAMEWORK

With the FCUP Laboratory Safety Manual (MSL), the aim is to consolidate good conduct practices and establish standards for new members.

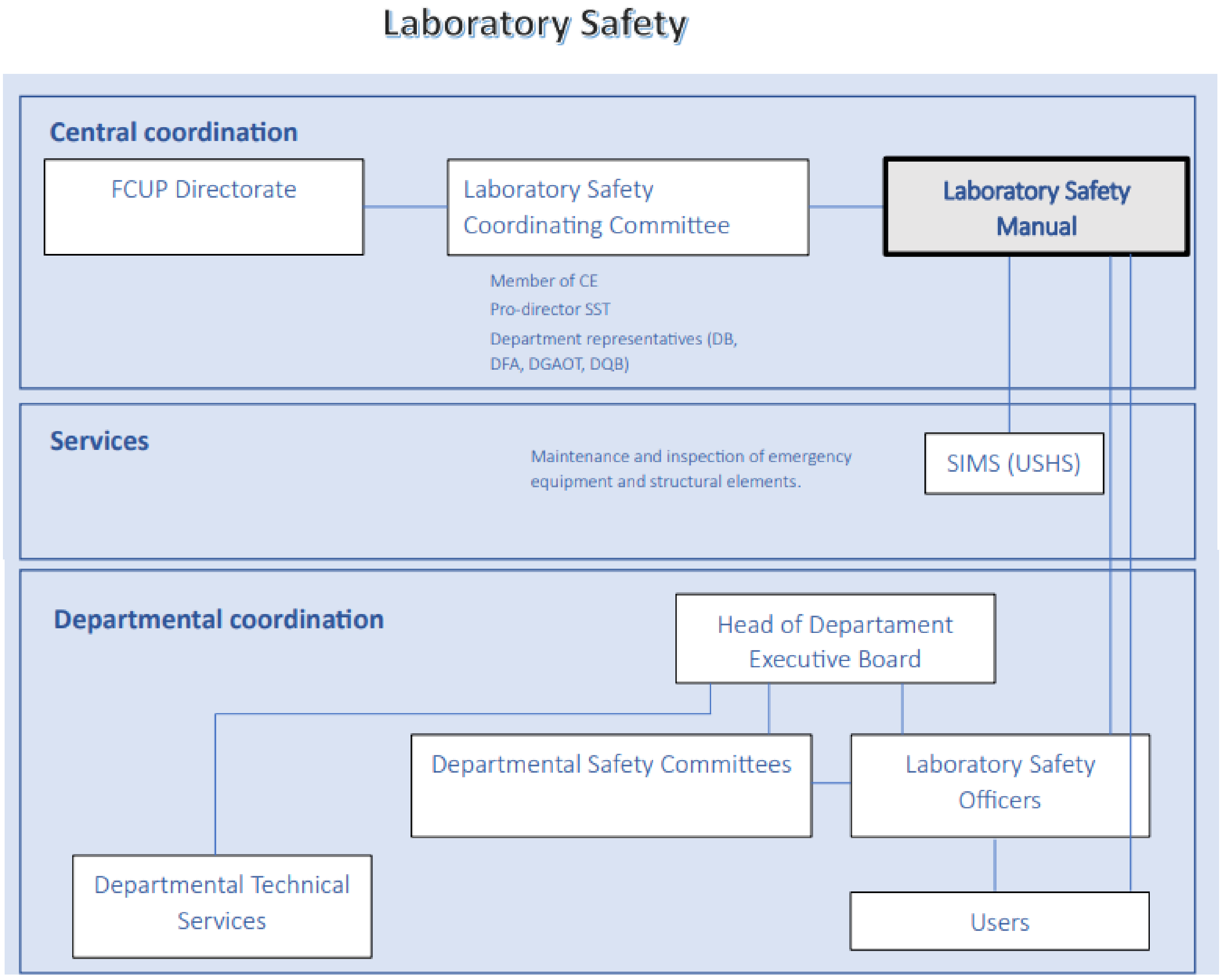
The MSL is a dynamic document subject to regular review, with a suggested minimum annual revision during the first phase of implementation. All laboratory users are invited to propose improvements to the MSL, for which they should contact the Laboratory Safety Coordinating Committee (ccsl@fc.up.pt), or the Departmental Safety Committees.

Addenda will need to be attached to the MSL for specific cases of laboratories that present other risks not covered here. The addenda should be drafted by the safety officer of that laboratory or group of laboratories. The set [MSL + addenda] will be identified in this document by a **specific MSL**. This should be submitted to the CCSL for verification before proceeding to approval by the FCUP Board.

MSLs (specific or otherwise) must be made available in Portuguese and English.

1.2 ORGANIZATIONAL STRUCTURE AND RESPONSIBILITIES

The organizational structure is based on this manual and is outlined in the following organization chart. This section also describes the responsibilities of each entity involved.



Directorate of FCUP

Institutes and approves the Laboratory Safety Manual (MSL), in its generic version applicable to the entire FCUP, and ensures the material and organizational conditions necessary for laboratory activities to proceed according to the guidelines stipulated in the MSL.

Intervenes in situations of violation of safety rules, deciding or delegating the decision on corrective measures, which include the possibility of inhibiting access to the laboratories.

Laboratory Safety Coordinating Committee (CCSL)

Drafts the MSL (in Portuguese and English) and keeps it updated, upon approval by the Directorate.

Ensures a harmonized implementation of the MSL in the different laboratories of FCUP, in coordination with the Departmental Safety Committees.

Establishes a post-implementation monitoring system, including the recording and analysis of occurrences, coordination of internal and external audits and inspections.

Supports the Departments and Safety Officers of the laboratories in complying with the legal requirements regarding the management of laboratory waste, coordinating the management of waste in an integrated way at FCUP, the hiring of certified companies for the collection of hazardous waste and the management of the SILiAmb platform.

Develops, implements and promotes training/information programmes.

Services of Infrastructure, Maintenance, and Sustainability (SIMS)

Ensures maintenance/inspection of firefighting and hazardous product spill equipment (extinguishers, safety showers, fire blankets, hoses, sandboxes, etc.), as well as the maintenance of structural elements of spaces and other equipment, as specified in this MSL.

Provides prior opinion on any changes to infrastructure or installation of equipment, even in cases where such interventions are carried out by Research Units.

Directors/Executive Committees of Departments

Implement, at the level of laboratories under their management, the safety organization established centrally, mobilizing the necessary means and human resources, including:

-Appointing Departmental Safety Committees;

-Appointing Laboratory Safety Officers (RSL) for the laboratories under their management;

-Requesting that Research Units managing laboratories in their facilities appoint the corresponding Laboratory Safety Officers.

Departmental Safety Committees

Support Departments and Laboratory Safety Officers:

-In identifying and assessing risks;

-In training/informing laboratory users;

-In implementing the MSL and adapting it to the specificities of each laboratory.

Develop and conduct training programs suitable for the specificities of the laboratories under the respective Departments' management.

Put into practice the post-implementation monitoring system, including recording and analyzing occurrences, conducting audits and internal inspections.

(Consult the Departmental Safety Committees **here**).

Laboratory Safety Officers (RSL)

Coordinate the supplementation of the MSL with specific information for each location (if applicable):

-Creating safety instructions for handling equipment (these instructions may be drafted by users under the supervision of the RSL. The information to be included should preferably be taken from the equipment instruction manuals);

-Ensuring the inclusion of information about any other relevant risk not addressed in the generic MSL, and the respective control measures.

Establish the way to ensure knowledge of the specific MSL (which should be recorded in a **commitment declaration**), and its compliance by all users of the laboratory.

Establish ways to ensure regular educational action regarding the risks associated with a particular task and respective control measures, and monitoring of their application in practice.

Report or appoint a third party to report accidents/incidents to the respective CS, and collaborate in their analysis.

Report or appoint a third party to immediately report any malfunction, damage, or disappearance in the safety infrastructures present in the laboratory (showers, eyewash stations, extinguisher, extraction, smoke detectors, etc.) to the Service of Infrastructure, Maintenance, and Sustainability (SIMS).

Consult or appoint a third party to consult the SIMS about any infrastructural change they intend to carry out in the laboratory. These cannot, among other restrictions, compromise the emergency plan in force against fire and other risks.

Note: the same RSL may be responsible for a set of laboratories, and a laboratory may have shared responsibility by several co-RSLs, to be established in an addendum in the specific MSLs.

Departmental Technical Services

Depending on the organization and nature of each department, the respective technical services may perform tasks related to laboratory safety, such as cleaning materials, autoclaving, disinfection, operationalization of waste management, storage of reagents, among others.

The performance of these tasks must proceed according to the procedures described in the MSL. In the case of tasks not mentioned in the MSL, the departments must contact the CCSL (ccsl@fc.up.pt).

If the technical services perform tasks characterizable as a user, for example performing services for external entities or supporting research, the technicians involved are also subject to the responsibilities and training plan of Users.

Users

May work in different laboratories provided they commit in writing to know and comply with all the specific MSLs that apply.

Must know the location of the MSL and know how to access safety data sheets.

Must plan and conduct each action in accordance with the instructions given by the RSL.

Report to the RSL events or conditions that they perceive as hazardous.

Carry out the safety training indicated by FCUP or RSL.

Keep all existing safety infrastructure in laboratories (e.g. fire-fighting equipment and first aid infrastructure) in the designated locations and completely unobstructed.

Be familiar with the procedures to be adopted in an emergency situation.

1.3 Training Plan

Safety and Emergency Procedures

The Self-Protection Measures (MAP) approved for FCUP, under the Fire Safety in Buildings regulation (SCIE), establish the delivery of training whose recipients are all FCUP staff not belonging to the Security Service, also created under SCIE.

The components of this training are:

- Compliance with prevention plan procedures;
- Compliance with alarm procedures;
- Compliance with general emergency action procedures, particularly evacuation procedures;
- Internal procedure in the event of an accident/incident.

Additionally, for laboratory users, it also includes:

- Familiarization with the spaces and identification of respective fire risks;
- Instruction on basic techniques for using first intervention means, namely portable fire extinguishers;
- Action in the event of a major accident, medical emergency, and minor consequences.

Laboratories

In the context of laboratory classes, teachers will be responsible for providing, in advance, students with all the necessary safety and hygiene training to carry out the practical activities of the respective courses (see Application in Class Context).

In any other context, laboratory users must be locally trained by someone experienced, designated by the RSL or supervisor, based on the MSL. This on-site training is the main solution for research and service laboratories.

The CCSL or other internal or external units to FCUP may offer other types of specific training in the future.

Each training action must be recorded in an individual training record (**FFI**). The actions that must necessarily be included in the FFI are:

- Check-In (knowledge of the spaces, access method, space assignment, general operating rules, dining areas, etc.);
- Information about the MSL and collection of the **commitment declaration**;
- Practices to be adopted regarding solitary work and conducting unsupervised experiments;
- Effective knowledge of the laboratory regarding the location of fire-fighting means, emergency exits, spill containment kits, etc.;
- Practices adopted in identifying and labeling materials, reagents, samples, waste, etc.;
- Management of all hazardous waste produced;
- Safe use of each of the necessary equipment;
- Practices adopted in the prior preparation of a new experimental job;
- Obtaining appropriate PPE and its correct disposal.

(mandatory in case of biohazard)

- Notions of the use of autoclaves and biological/laminar flow safety chambers;
- Basics of laboratory safety and handling, transport and storage of microorganisms / cell cultures / GMOs;
- General notions and decontamination of material or waste with biorisk;
- How to act in case of exposure, with risk of infection, and in case of spillage of biological agents.

(mandatory in case of major physical risks)

- Use of electrical voltages greater than 500 volts not limited to maximum currents of less than 1 milliampere;
- Use of lasers and other optical sources of class above 2;
- Handling and transport of compressed gases and cryostatic liquids.

(mandatory in case of the existence / use of hazardous substances)

- Local organization of the storage, access and transport of hazardous substances;
- Location and consultation of safety data sheets;
- Handling and storage of flammable liquids;
- Handling and storage of potentially explosive compounds;
- Procedures for the use of fume hoods and/or glove boxes;
- Practical instructions on the use of TACMR / completion of the TACMR use approval form;
- Handling and storage of corrosive compounds.

Staff training should be reformulated or strengthened when new risks are introduced into their workplace, or by assignment to different workplaces involving new risks or protective measures.

1.4 Application in Class Context

The MSL is applicable to classroom labs, although specificities that require adaptations are recognized.

Department

The Department designates a RSL for the classroom labs, and provides all the necessary conditions for compliance with the established safety rules. In the case of PPE, the Departments may maintain the practice of requiring students to bring their PPE considered necessary for their protection (e.g. lab coat, goggles, etc.).

RSL

The classroom labs must have a RSL, designated by the Department, which coordinates the adaptation of the MSL to their specificities, namely the inclusion of safety instructions for handling the equipment and any other relevant risk not addressed in the generic MSL, and the respective control measures. They report or designate a third party to immediately report to the Infrastructure, Maintenance and Sustainability Service (SIMS) any breakdown, damage or disappearance in safety infrastructures. They consult or designate a third party to consult SIMS about any infrastructural changes they intend to make in the laboratory.

In this case, the RSL establishes how to ensure and document the knowledge of the specific MSL by all teachers and staff who use the classroom lab in question.

Teachers in a class situation

Teachers are responsible for:

- Ensuring the availability in advance of all safety and hygiene indications necessary to carry out practical activities, in coherence with the MSL;

- Ensuring a regular pedagogical action regarding the risks associated with a given task and respective control measures, and monitoring of its application in practice;

- Reporting accidents/incidents to the respective Safety Committee, and collaborate in their analysis;

- Ensure leadership in responding to an emergency situation (e.g., evacuation).

Practical laboratory classes should not, except in well-founded cases, take place with safety requirements lower than those stipulated in the general MSL.

Students

They must follow all the safety and hygiene instructions necessary for the execution of practical activities, which must be included in instructions made available in advance.

They must report to the Teacher events or conditions that they perceive as hazardous.

They must keep all existing safety infrastructure in laboratories (e.g. fire-fighting equipment and first aid infrastructure) in the designated locations and completely unobstructed.

They must be familiar with procedures in an emergency situation.

Management of high biological, physical and chemical risk

In general, the following should not be used in practical classes:

- [Biological risk]: biological agents with a risk level greater than 1 or with an unknown risk;

- [Physical hazard]: work in high-energy, high-pressure, exposed energized electrical systems, lasers class 3R and above, cryogenic fluids, pressurized or vacuum products handled by students;

- [Chemical hazard]: CMR substances, substances of acute toxicity cat. 1 and 2 (cutaneous and aerial routes), or potentially explosive.

Exceptional exposure to the above hazards is subject to the following standards:

a. It will always be adjusted to the existing means (infrastructures, standardized operating procedures of the laboratory and its applicability, and individual protection);

b. Students must previously possess the necessary skills, acquired in specific prior training, through practical training carried out using innocuous conditions that simulate high-risk conditions. For example, a student will only be able to handle a mutagenic liquid after acquiring specific knowledge about the handling of such liquids, waste disposal, spill cleanup, interpretation of SDS, use of PPE appropriately, use of general protective equipment, such as fume hoods, and use of emergency equipment, such as eyewash stations.

c. A written request must be made by the course regent addressed to the RSL, including:

  i. A copy of the class protocol;

  ii. Declaration that all of the following conditions are met:

    - The pedagogical importance;

    - The absence of a less hazardous alternative;

    - The existence of infrastructural, instrumental and personal safety conditions for said activity.

Courses with high-risk activities.

These courses should include in their programmes basic notions about the risks involved. The protocols of the practical classes should include information, consistent with the specific MSL, on risk assessment, handling and disposal of the products involved in each class.

The risk should be assessed according to the maturity and level of education of the students. The protocols of the 1st and 2nd year of the Bachelor's Degree must also include a detailed description of:

i. Standard operating procedures to be used in each class;

ii. Guidelines on the use of equipment, substances or organisms to be used in each class. The class protocol must specifically refer to the hazardousness of all these elements and the procedures for their safe handling.

1.5 AUDITS AND INSPECTIONS

The description of the procedures for audits and inspections will be dealt with at a later stage than the implementation of the MSL. They will be essential tools for monitoring the effectiveness of the MSL.

2.1 ACCESS AND EXECUTION OF WORK

Normal Opening Hours

For the purposes of safety regulations, the normal opening hours of FCUP laboratories coincide with the **opening hours of the respective buildings**.

Categorization of work outside normal working hours and its regulation

Work done outside of normal operating hours is not permitted for pre-graduate students.

The remaining users are subject to constraints depending on the risk category (whenever you have doubts, consult the Departmental Safety Committee):

Typology of work	Applicable constraints
Category 1- Any laboratory work that the supervisor or RSL deems does not endanger the operator and the facility.	Can be performed outside of normal hours.
Category 2- Any laboratory work involving risks above category 1, and which does not involve the type of operations described in category 3.	It can be done outside of normal hours, but not in solitary mode (see Solitary work).
Category 3-Any laboratory work that involves the following situations: - Work on high energy, high pressure or exposed energized electrical systems; - Work with potentially explosive, high-fire risk (e.g. any work with a live flame), highly toxic and/or fast-action chemicals; - Transfer of flammable or cryogenic liquids, except in very small quantities (< 1L); - Similar works to the previous ones	Cannot be held outside of normal operating hours At normal hours, it cannot be performed in solitary mode. (see Solitary work)

The details regarding access to the laboratories outside normal hours, and their articulation with the safety post of the respective building, must be established in specific documents, separate from the MSL.

Solitary work

It is considered in this document that solitary work happens whenever an individual performs activities while not in audio or visual contact with another trained and experienced individual.

Solitary work can present a considerable or unacceptable risk, even when performing category 1 work. This is particularly true for people with a propensity for sudden illness or disabling state, either physically or mentally. Persons identified in this group should never carry out solitary work, unless a regular verification system is maintained, at least every 30 minutes (by telephone or face-to-face).

In the case of the presence of minors, for example in Summer Schools or Junior University, permanent monitoring by a supervisor is mandatory.

Work considered category 2, when performed by pre-graduate students, should never be carried out in solitary mode, and never outside normal operating hours.

Experiences taking place without human presence

Only experiments classified as category 1 or 2 may be carried out without human presence, provided that an RSL authorizes it. In such a case, the following shall be complied with:

- The laboratory light should be left on for the purpose of the safety patrol;
- Notices should be placed identifying the person responsible in the event of an emergency, the nature of the experience, and the hazards involved;
- The RSL or supervisor shall inform the respective safety post [seguranca.fc3@fc.up.pt (FC2/3), seguranca.fc4@fc.up.pt (FC4/5)] by email that there is an ongoing experiment;
- Where appropriate, arrangements shall be made for other users to periodically inspect the operation.

All experiments involving hazardous chemicals should be carried out in fume hoods.

Floods are one of the most frequent occurrences, and are mostly avoidable. All pipe connections must be fixed (with clamp or similar). Additionally, it is highly recommended to use safety systems that monitor water or gas flows, and that allow electrical equipment to be turned off.

Note: experiments are not understood here to mean cases in which there is simply equipment that is permanently switched on, such as growth chambers, freezers, incubators, etc. However, the **General Practices for Safely Working with Equipment** apply to this equipment.

1. GENERAL ORGANIZATIONAL ASPECTS

2. ACCESS AND RISK MANAGEMENT

ACCESS AND EXECUTION OF WORK

GENERAL RULES

BIOLOGICAL RISK
BASIC CONCEPTS
GOOD PRACTICES
PARTICULAR CASES

PHYSICAL RISK
ELECTRICAL RISKS
ELECTROMAGNETIC RADIATION
NOISE
CRYOGENIC FLUIDS
COMMON EQUIPMENT

CHEMICAL RISK
GENERAL PREVENTIVE MEASURES
PURCHASE OF HIGHLY HAZARDOUS CHEMICALS
STORAGE
SAFE TRANSPORT
FIRE PREVENTION
POTENTIALLY EXPLOSIVE COMPOUNDS
PARTICULARLY HAZARDOUS SUBSTANCES (HEALTH)
CORROSIVE SUBSTANCES
COMPRESSED GASES

LABORATORY VENTILATION

PERSONAL PROTECTIVE EQUIPMENT

HAZARDOUS WASTE MANAGEMENT
CHEMICAL WASTE
BIOLOGICAL WASTE
RECYCLING
RADIOACTIVE WASTE

3. ACCIDENTS AND EMERGENCIES

ABBREVIATIONS

CREDITS

ATTACHMENTS

2.2 GENERAL SAFETY RULES

The basic rules described in this section comprise good practices that apply to most laboratories. In the sections on **Biological Risk** and **Chemical Risk** are specific rules for laboratory work where these risks are augmented.

- It is forbidden to eat, drink, smoke, apply cosmetics, put on/remove contact lenses, or store food for human consumption in laboratories;

- The use of Personal Protective Equipment (PPE) appropriate to the work to be performed is mandatory. See more details in **Personal Protective Equipment**;

- Avoid touching your nose, mouth or other mucous membrane with your hands, as well as not putting objects in your mouth;

- Long hair should be tied up and it is not recommended to wear accessories such as rings and bracelets;

- The use of headphones in the workplace should be restricted to the minimum and to work functions;

- Experimental work should be planned in good time and all associated risks should be identified in order to adopt procedures to minimise them. It must also be verified that the appropriate conditions exist for the collection of the waste produced, complying with the rules of procedure set out in **Waste management**;

-Hand washing should be done before and after a procedure has been performed, and before leaving the laboratory. It should be repeated as often as necessary, including before and after putting on gloves and whenever coming into contact with contaminated materials/surfaces;

- The pipettors available in the laboratories should be used, and it is forbidden to pipette with the mouth.

- The use of sharps (e.g., syringe needles and scalpels) should be limited to what is essential. Needles should be disposed of in a sharp-piercing container;

- All laboratory material, with or without contamination, should be disposed of in an appropriate container and never in the common garbage (see more information in **Waste management**);

-In each laboratory, there must be procedures for cleaning and maintaining the equipment, according to its specificity, and in order to ensure its proper functioning. Maintenance records shall be kept and any anomalies shall be reported to the RSL;

-The laboratory must be kept tidy, clean and free of materials that are not necessary for its activity. Computer work, paper record keeping, etc., should be carried out in an area of the laboratory separate from the areas where hazardous material is handled.

1. General organizational aspects
2. Access and risk management
Access and Execution of Work
General Rules
Biological Risk
Basic concepts
Good practices
Particular cases
Physical Risk
Electrical risks
Electromagnetic radiation
Noise
Cryogenic fluids
Common equipment
Chemical Risk
General preventive measures
Purchase of highly hazardous chemicals
Storage
Safe transport
Fire prevention
Potentially explosive compounds
Particularly hazardous substances (health)
Corrosive substances
Compressed gases
Laboratory Ventilation
Personal Protective Equipment
Hazardous Waste Management
Chemical waste
Biological waste
Recycling
Radioactive waste
3. Accidents and Emergencies
Abbreviations
Credits
Attachments

2.3 BIOLOGICAL RISK

2.3.1 BASIC CONCEPTS: BIOLOGICAL RISK AGENTS AND SAFETY LEVELS IN THE LABORATORY

Biological agents are cells or derived material endowed with the capacity to reproduce, to transfer genetic material (bacteria, viruses, fungi and parasites, genetically modified material, cell cultures), susceptible to causing infections, allergies or resulting products capable of causing poisoning.

Biorisk Level (BRL) and Biosafety Level (BSL)

Biological agents are classified into four risk levels (BRL-1 to 4), according to the level of infectious risk to humans (Decree-Law No. 102-A/2020 which transposes EU Directives 2020/739, 2019/1833, 2000/54 into national law).

Safety measures should be applied according to the nature of the activities, assessment of the risk to workers and the nature of the biological agent in question – Biosafety Levels (BSL-1 to 4).

Summary table of Biorisk levels and respective means of Biosafety/Containment:

Group #	Risk description	Propagation risk	Biosafety Levels (BSL) and Containment
BRL-1	Agents with a low probability of causing disease in humans.	No risk of spread (e.g. aerosols) and unnecessary treatment/prophylaxis.	**Biosafety Level 1 Laboratory (BSL-1)** Laboratory with basic requirements (e.g. waterproof benches, flow chamber, autoclave, etc.) for handling microbiology, other cells or agents with no associated risk.
BRL-2	Agents that can cause disease in humans, but with a small probability of spreading in the community, for which effective means of treatment exist.	Low risk of spread and there is treatment /prophylaxis.	**Biosafety Level 2 Laboratory (BSL-2)** Laboratory equipped with BSL-1 measures and with additional biosecurity measures including: - Biosafety Chambers (e.g. handling with risk of creating aerosols); - Display of biorisk information visible at the entrance (see symbol below); - Doors permanently closed; - Potentially contaminated waste treated and placed in containers other than the general waste system, with decontamination of waste and containers before being washed; - Safe storage of biological material (and registration with backup); - Staff with specific training
BRL-3	It includes agents that can cause disease in humans and are likely to spread in the community, although prevention or treatment is possible.	Risk of spread and treatment/prophylaxis is available.	**Biosafety Level 3 Laboratory (BSL-3)** Laboratory equipped with BSL-2 measures, and with additional biosecurity measures that include: - Prior authorization of manipulation by the responsible regulatory entities and the institution (submission of project, period, demonstration of physical conditions and human resources, etc.); - Isolated laboratory with antechamber access; - Laboratory with its own equipment (e.g. autoclave for decontamination of waste before disposal; washbasin with sensor controls); - Laboratory with controlled negative pressure, and air inlet and outlet devices with HEPA filters or equivalent; - Fumigation and periodic and controlled disinfection; -Certification and regulated maintenance of all handling in biosafety chamber and all equipment to be used.
BRL-4	Agents that can cause serious diseases in humans and present a high risk of spreading in the community, for which there are no effective means of treatment.	High risk and/or no treatment/prophylaxis.	**Biosafety Level 4 Laboratory (BSL-4)** Maximum Containment Laboratory - Level 4 - equipped with measures applicable to BSL-3, and with additional biosecurity measures that include: - Special authorization in addition to the authorizations requested for BSL-3; - Operation under the supervision of national health authorities or equivalent bodies; - Requirement of exceptional infrastructures; - Only trained personnel.

The biohazard symbol (see below) must be affixed to the door of class and/or research laboratories in which BRL-2 microorganisms are handled - see attachment for more details.

Important measures at FCUP

Regarding biosafety measures, Laboratory Safety Officers (RSL) must follow the guidelines set out in the World Health Organization's Manual of Biological Safety in Laboratory, and the European Union Directives transposed into national legislation.

Laboratories for the handling of biological agents must follow rules of individual and collective protection (e.g. be equipped with emergency showers, fume hoods, biosafety chambers, hazardous waste containers, etc.).

Several FCUP laboratories are properly equipped with primary and secondary barriers that allow the manipulation of biological risk agents 1 or 2 in these laboratories, while the manipulation of biological risk agents 3 and 4 is not authorized.

For cases in which biological risk agents 2 are used for the first time, the RSL must inform a departmental representative of the CCSL and the Head of Department of the start of the activity (agent involved, the procedures to be adopted and who ensures the necessary conditions). After this information, the RSL must initiate the mandatory procedures: notification of the Authority for Working Conditions (ACT) and the Directorate-General for Health (DGS) at least 30 days in advance of the start of activities. The notification is made in an appropriate model for the computer processing of data, provided by ACT and DGS. Likewise, the legally required updating of the information regarding these activities is the responsibility of the RSL.

Risk 2 biological agents may only be handled in work areas corresponding to biosafety level 2 or higher (BSL-2 Laboratories), and the following requirements shall be considered:

- All users/workers potentially exposed to BRL-2 agents (including researchers, teachers, students and laboratory technicians) must have basic training considering the type of agent, the degree of risk, safety measures to be adopted and type of work to be performed;

- In BSL-2 laboratories, it is required to display visible biorisk information at the entrance, with indication of the RSL;

- Access to BSL-2 laboratories and their equipment is limited to authorized members, and the rules of the previous table must be followed;

- The RSL is the one who evaluates each case and determines who has access to and/or can work in the laboratory, as well as the minimum level of protective clothing to be used;

- The Biosafety Officer (Departmental SC) must have up-to-date information on the BSL-2 organisms (and storage site) used in the teaching and research laboratories;

- - In case of lack of knowledge, the Precautionary Principle must be followed and biosecurity measures of a level equal to or higher than BSL-2 must be adopted.

In addition, the available lists of biohazard agents, such as:

-The list of biological agents known to be infectious to humans, contained in Annex V of DL 102/2020 – this DL excludes Genetically Modified Organisms (GMOs) / Genetically Manipulated Microorganisms (GMMs), and organisms that do not cause known diseases to humans, but have a harmful effect on the environment, including plants;

- For consultation on GMOs, see Decree-Law No. 115/2015;

- For consultation on animal handling, see Decree-Law No. 113/2013 (animal handling section);

- http://atcc.org/ - this or other websites (e.g.: https://wi.knaw.nl/page/Collection) of collections of microorganisms and animal cells that classify each strain/lineage according to the level of biosecurity required;

- https://www.eppo.int/ - website that informs about quarantine organisms for plants (list A1 and A2);

- https://www.dgav.pt/animais/conteudo/animais-para-fins-cientificos/.

2.3.2 GOOD PRACTICES FOR HANDLING BIOLOGICAL RISK AGENTS

General Rules

The basic rules described below comprise good practices in laboratories working with microorganisms, cell cultures, and M/GMOs. Their application aims to minimise exposure to biohazards and prevent accidents, and complements the rules described in General Safety Rules. Some procedures are described in more detail in other sections of this manual.

The handling of animals or microorganisms/genetically modified organisms (GMOs) requires these and other additional procedures, described below:

- All biological material must be properly identified/labeled;

- It should be ensured that appropriate source control measures are used for the organisms being tested. Work under aseptic conditions can be done in flame for BRL-1 bacteria, but has to be done in biological safety chambers (BSCs) for BRL-2 microorganisms, filamentous fungi, cell cultures, and O/MGMs;

- Laboratory doors and windows must remain closed, in particular when work is being carried out in flame or CSB (see more information in Laboratory ventilation);

- Work surfaces should be disinfected before and after performing any procedure, as well as at the end of the day. Preferably 70% ethanol should be used, which should always be available in all laboratories;

- The transport of biological material must be done carefully, preventing the risk of spills and other accidents;

- All work, in particular the handling of liquid cultures, must be carried out in such a way as to avoid the formation of aerosols and droplets, as well as to prevent spillage. Aerosols have an associated risk of inhalation and, once deposited on surfaces, the risk of ingestion or contact with mucous membranes increases. Work with a risk of aerosol formation should therefore be carried out in CSB;

- In the event of a spill of biological material, the affected surfaces should be properly disinfected, preferably with 20% bleach (see more information in Procedure in emergency situations).

Note: *In this manual, the bleach concentration refers to the percentage of dilution from the commercial product (bleach with active chlorine concentration between 25 and 50 g/L).*

Hand washing and surface disinfection

Hand washing should be done before starting work, before putting on gloves and after they are removed, after handling biological material, whenever coming into contact with contaminated material/surfaces, and before leaving the laboratory. It should be repeated whenever necessary, and should be done according to the procedure described below (whenever possible make diagrams of the procedures available in the laboratory). Before leaving the laboratory, hand washing should be followed by disinfection with Alcohol-Based Antiseptic Solution (ABAS) (**see details as attachment**). Work surfaces should be disinfected before and after any procedure, as well as at the end of the day, and whenever there is a spill (**see details as attachment**).

Transport of biological material

The transport of biological material with associated biohazard must be done in such a way as to avoid accidental spillage. To this end, intra-laboratory transport (e.g. from the bench to the incubator) must be done using trays, taking particular care with the transport of glass material. Circulation outside the laboratory should be limited to the essential minimum, but, when unavoidable, interlaboratory transport should be done in closed containers, and transport between FCUP buildings should be done inside thermal bags (triple pack). Used containers must be properly decontaminated to be transported without gloves. Whenever possible, opt for the use of carts and avoid carrying them by hand.

Personal Protective Equipment (PPE)

This equipment aims to protect the user from exposure to biological hazards and must therefore be suitable for the work to be carried out, taking into account the respective risks. They should be used correctly, in conjunction with good laboratory practice (and never in place of it). The use of PPE should be restricted to laboratory areas, avoiding circulation with them outside the laboratory.

More information at **Personal Protection Equipment**.

2.3.3 Particular Cases

2.3.3.1 Animal Handling

Manipulation, the 3Rs principle, and regulation

"*Animal manipulation*" refers to experimentation procedures carried out on live animals for the purpose of research in basic or applied biology (e.g. evaluation of the efficacy of new medicines and testing of human health and/or environmental safety of consumer and industrial, pharmaceutical and industrial/agrochemical products), or advanced education or training in this field.

The handling and housing of animals for experimentation/teaching must follow:

- The BRL and BSL principles defined earlier;
- Specific procedures of the European animal handling regulations (e.g. Directive 2010/63/EU and Regulation (EU) 2019/1010);
- Principle of the 3Rs (Replacement, Reduction, Refinement) - regardless of whether the purpose is for research or teaching, it must follow what is defined in Decree-Law No. 1/2019.

In education, preference should also be given, in addition to reduction, to artificial and in silico models, or to the use of commercial cadavers. When euthanasia/occupancy is necessary, these procedures should follow the humanitarian principles of non-suffering (3Rs).

Regulated Animals

The legislation in force in Portugal is Decree-Law No. 113/2013 (which transposes **Directive 2010/63/EU**), updated by Decree-Law No. 1/2019, which requires the existence of a Body Responsible for Animal Welfare in establishments where animals are raised/used for scientific or educational purposes, compliance with the principle of 3Rs, and the classification of severity and annual reports on the number of animals used in each institution (and the severity of the procedures).

Both European and national legislation require minimum conditions for housing (e.g. vivarium) and care in animal experimentation/manipulation (in Portugal projects involving manipulation require DGAV evaluation), namely the assessment of pain, suffering and lasting damage caused to animals. Specialised user training and regular inspections of the premises are required.

It should always be preferred to work with more distant taxonomic groups. For the use of embryonic forms of birds (e.g. CAM assay), consideration should be given to the regulation of the developmental periods from which handling requires authorisation.

"Animal under protection" in animal experimentation/manipulation states that *"embryonic and fetal forms of mammals, birds and reptiles are protected during the last third of their gestation or incubation period, fish and amphibians as they can feed independently, and cephalopods at the time they hatch. Embryonic and foetal forms are protected from an early stage of development if they live beyond the stage described above and the procedure is likely to cause them pain, suffering, distress or lasting harm after they have progressed to that stage* (***Animals (Scientific Procedures) Act 1986***).

The following websites can also be consulted:

- **https://www.dgav.pt/animais/**;
- **https://environment.ec.europa.eu/topics/chemicals/animals-science_en**.

Risks and risk management

When working in an animal handling laboratory, additional care must be taken to those mentioned above, due to the risks associated with the characteristics of the animal itself and/or the agent used in its handling (e.g. escape, bite, allergies, etc.).

The laboratory must be equipped with primary and secondary containment measures.

Overpopulation, inappropriate contact of competitive specimens, etc., should be avoided, and these practices are framed in the 3Rs Principle.

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In addition, if animals are infected with pathogens, they may cause a risk to the user and/or other animals, so risks associated with zoonotic agents/infections, or posing a risk to the environment, should be recorded and prevention measures strictly followed.

Important measures at FCUP

FCUP does not currently have the infrastructures or human resources that allow the respective authorization for long-term animal handling (e.g. vivarium), so it sometimes resorts to the supply of animals by vivariums of the University of Porto (UP). Likewise, FCUP does not currently have the infrastructure or human resources to allow the handling of vertebrate or invertebrate animals that have a BRL above 1.

In addition, animal manipulation at FCUP, both for research and teaching purposes, is highly limited to distant taxa animals and early embryonic forms.

Teaching at FCUP that involves the use of regulated animal models is restricted to the use of commercial cadavers or those provided by vivariums of the University of Porto (UP). Other models used involve animals not included in the definition of animal manipulation (e.g., *Insecta* or *Annelida*). Euthanasia/ocision of regulated animals for teaching purposes is not permitted. The use of bird embryos for experimentation is allowed only for the first stage of development, which does not involve suffering.

In general, Decree-Laws No. 113/2013 and No. 1/2019 (which transpose **Directive 2010/63/EU**) must be complied with, and the RSL is in charge of informing the departmental representatives of the CCSL, ensuring the proper training of laboratory members, compliance with the conditions for animal handling, and carrying out notifications and information with the entities (e.g., DGAV). It is also necessary to keep an annual register of risk assessments of activities involving the use of this type of material.

Notes: FCUP supports the Aquatic Animal Sciences Laboratory Course (CAL-AQUA) in partnership with the Interdisciplinary Center for Marine and Environmental Research (CIIMAR) of UP; UP offers training courses for animal experimentation technicians at multiple levels.

2.3.3.2 Animal Cell Cultures

Mammalian cell culture is a set of techniques that allow the growth of tissue fragments from different mammalian species in an *in vitro* environment to study, for example, cell behavior, maintaining its properties (physiological, metabolic, biochemical, genetic, etc.), or for regenerative purposes. Cells can come from primary cell cultures (cells obtained from human tissue or other animals) or immortalized cell lines. Many cell lines are acquired from collections (e.g., ECACC and ATCC).

There are cell lines with different levels of biorisk. If a low-risk biohazard cell line comes into contact with an agent (e.g., virus) that is of a higher biohazard, the culture has the biohazard level of that agent.

In addition to the rules of manipulation in the laboratory mentioned above, human/animal cell culture laboratories require particular training in equipment and procedures (e.g. CO_2 incubator, cryopreservation and cell retrieval). It should also be noted that:

- Whenever the work is finished, the entire surrounding area must be disinfected (e.g. with 70% ethanol);
- For the elimination of cells, 70% ethanol (or bleach with a final dilution of 10%) should be placed and left to act for 30 minutes, or alternatively, place in containers and autoclave for at least 30 minutes;
- The external surface of tubes, media vials, plates, etc., must be decontaminated by passing paper soaked in 70% ethanol before placing the material in any equipment (e.g. centrifuges, incubators, etc.);
- Care must be taken to prevent spills and aerosol production;
- Do not keep hands, pipettes or other devices on the work surface;
- Particular care should be taken when agitating BRL-2 material, in addition to ensuring that equipment such as centrifuges, biosafety chambers and incubators are restricted to animal cell cultures;
- In the event of a spill of liquids with a risk level BRL above 1, the spill must be cleaned with absorbent paper soaked in 70% ethanol or 20% bleach and disposed of in the biohazard waste container. In case of spillage in equipment (e.g. centrifuge), wear a mask during cleaning to avoid breathing aerosols;
- The use of equipment, such as CO_2 incubators, inverted microscopes, etc., requires prior training and careful use to avoid spillage, aerosol release or contamination of samples;
- Contamination of cell lines by other lines and/or mycoplasmas is frequent, so laboratories should periodically screen their animal cell cultures.

Important measures at FCUP

FCUP has some research and teaching laboratories dedicated to the manipulation and culture of primary and/or immortalized cell lines of risk level BRL-1 (for teaching) and up to risk level BRL-2 (for research).

RSLs should update biosafety officers (departmental CS) on the cell lines in use and stored (and their storage location) in both teaching and research laboratories.

In cases of manipulation of BSL-2 security level, an application for prior authorization must be made to the CCSL. If this is authorized, the responsible party must follow the legislation in force. An annual record of risk assessments of activities using this type of material shall also be kept.

2.3.3.3 Genetically modified organisms (GMOs) and genetically modified microorganisms (GMMs)

Definition

"*A Genetically Modified Organism (GMO) is any biological entity with the capacity to reproduce or transfer genetic material, except humans, whose genetic material has been modified in a way that does not occur naturally through interbreeding and/or natural recombination.*" "*Genetically modified microorganism (GMM) is a microorganism whose genetic material has been modified by a form of sexual reproduction and/or natural recombination that does not occur in nature*" (Decree-Law No. 55/2015).

Regulation

The rules for handling GMMs and GMOs at FCUP are governed by the provisions of Decree-Law No. 55/2015, which applies to their availability for use in confined-use activities, to limit their contact with the general population and the environment, and providing a high level of safety.

Are excluded from the scope of application of this Decree-Law (Annex 1 of Decree-Law No. 55/2015):

- Mutagenesis;
- Cell fusion (including protoplast fusion) of any prokaryotic species that can exchange genetic material through known physiological processes;
- Cell fusion (including protoplast fusion) of cells of any eukaryotic species, including the production of hybridoma and the fusion of plant cells;
- Self-cloning consisting of the removal of nucleic acid sequences from a cell of an organism, followed or not by the reinsertion of part or all of that nucleic acid (or its synthetic equivalent), possibly after enzymatic or mechanical treatment, into cells of the same species or cells of phylogenetically related species which can exchange genetic material with each other through natural physiological processes, and where the resulting microorganism is not likely to be pathogenic to humans, animals or plants; Self-cloning may include the use of recombinant vectors where extensive experience of safe use is available in the microorganism concerned.
- GMMs that cumulatively meet the criteria listed in paragraph 2 of Annex I to the aforementioned Decree-Law, relating to the safety of GMMs for human health and the environment, i.e. where the authenticity of the strain (and the known and verified modification) is verified, and for which there are documented safety and genetic stability data (absence of pathogenicity, toxicity, allergenicity or any other harmful agents, including to the environment, or risk of transfer of genetic material).

The following are considered genetic modification techniques, among others (Annex II of Decree-Law No. 55/2015):

-Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by inserting nucleic acid molecules, produced by any method, outside an organism, into any virus, bacterial plasmid or other vector system and incorporating them into a host organism where they do not exist naturally but in which they are capable of continued propagation;

-Techniques involving the direct introduction into a microorganism or organism of hereditary material prepared outside of that microorganism or organism, including microinjection, macroinjection and microencapsulation;

-Cell fusion or hybridization techniques in which there is formation of living cells with new combinations of hereditary genetic material, through the fusion of two or more cells by mechanisms that do not occur naturally.

The category of GMOs does not include those resulting from techniques that involve the direct introduction of hereditary material into an organism (as long as they do not involve the use of recombinant DNA/RNA molecules or GMOs), including in vitro fertilization, conjugation, transduction, transformation, polyploid induction and any other natural process (Annex II of Decree-Law No. 55/2015).

A pure, chemically defined substance obtained through biological processes and which does not contain GMOs, heterologous proteins, or recombinant DNA is not included in the category of GMO derivative.

The contained uses of GMOs are organised into classes, which correspond to the levels of containment considered necessary for the protection of human health and the environment, namely:

- «Class 1», zero or negligible risk operations, where a level 1 containment is sufficient;
- «Class 2», low-risk operations, where a level 2 containment is required;
- «Class 3», Moderate-risk operations, where a Level 3 containment is required;
- «Class 4», high-risk operations, in which a level 4 containment is required (Art. 7 of the aforementioned DL)

Important measures at FCUP

Currently, only operations with GMOs excluded from the scope of Decree-Law No. 55/2015 (see above) or classified as level 1 are authorized at FCUP, upon request to the Portuguese Environment Agency (APA, I.P.), and respecting the following conditions:

i) the microorganism or recipient or parental organism is unlikely to cause disease in humans, animals or plants;

ii) the MGM or GMO is unlikely to cause disease in humans, animals or plants and to have adverse effects on the environment;

iii) the nature of the vector and the inserted element does not give rise to a GMM or GMO with a phenotype likely to cause disease in humans, animals, or plants or to give rise to adverse environmental impact (Annex III to that DL).

<u>The use of transgenic material (e.g. plants) in a non-confined environment (e.g. field greenhouses) is not allowed</u>.

The biosafety officer (departmental CS) must have up-to-date information on the GMOs in use and stored (and their storage location), both in teaching and research laboratories.

Cumulatively with general safety practices, groups that intend to handle class 1 GMOs in FCUP (teaching/research) <u>should also consider practicing the general principles and containment measures provided for in Annex IV of Decree-Law No. 55/2015, so that exposure in the environment and in the workplace is kept at the lowest possible level and a high degree of safety is guaranteed</u>.

To ensure containment, good working practices, training and containment equipment, as well as the design of exceptional facilities, should be adopted. For all activities involving GMMs or GMOs, the principles of good laboratory practice in microbiology shall be applied, as well as the following procedures:

- Keep the level of exposure to any GMMs or GMOs, both in the workplace and in the surrounding environment, as low as possible by adopting control measures at source:
  - MGMs should be handled in biosafety chambers;
  - GMO plants must be kept in restricted greenhouses for this purpose (identify the growth chambers), limiting their handling to the antechamber of the greenhouse, namely their processing. Biological tissues to be analysed in laboratories must be transported in

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closed or preserved containers. There should be adhesive mats next to the doors. It is mandatory to affix the biohazard sign on the doors. Unwanted species such as insects, rodents and arthropods should be limited;

-In the chambers and antechambers of plant growth or handling of MGMs, gowns of limited use in these spaces should be used. These should be periodically autoclaved. Countertops must be easy to clean, and resistant to water, acids, bases, solvents, disinfectants and decontamination agents. Hand sanitizer and countertops should be made available;

- The keeping of genetically modified animals must also comply with all procedures relating to **Animal handling**;

- All solid waste generated in the handling of GMOs must be disposed of in appropriate waste containers and autoclaved (including plant remains, soil, solid GMM crops, gloves and disposable material);

- All liquid crops and liquid residues shall be decontaminated by adding bleach in a final dilution of 20 % for at least 30 minutes;

- Regularly test and ensure the maintenance of control measures and equipment, keeping records of checks and maintenance of handling and storage equipment;

- test, where necessary, for the presence of viable manipulated organisms outside the physical containment zone, including through the use of GMO detection kits and antibiotic susceptibility tests;

- Ensure adequate training of users, promoting training between researchers and students, and keeping records of such training;

- Maintain and update records of all GMOs and GMMs, which must be uniquely identified at growth and storage sites;

- In situations of dissemination of MGM proceed as exposed in **Accidents and spills of biological material** . Accidentally disseminated plant material should be autoclaved.

Groups wishing to carry out operations with GMOs classified in class 1 still have to:

-Submit to the respective Department and to the CCSL of FCUP the activity proposal, specifying the regulations and biosafety measures that will be adopted, identifying the risk class and ensuring the appropriate training of technicians, students, and other participants in the project;

- Submit to APA, I.P. the request for the use of GMOs in a confined environment in accordance with Decree-Law No. 55/2015;

- Review the risk assessment and containment measures on an annual basis, and any other protective measures adopted;

- Maintain an annual record of the risk assessments of the contained use activities carried out, which must be made available to APA, I. P., and other competent entities, whenever requested;

-Develop procedures related to accident prevention, action in the event of emergencies, user training and waste and effluent treatment, and for this purpose must draw up an emergency plan that includes the safeguarding of human health and the environment, to be adopted in the event of failure of the planned containment measures;

- Inform the bodies and entities likely to be affected in the event of an accident, about the emergency plans and the safety measures that must be applied, informing APA, I. P. of this information;

- Ensure, in accordance with the law, the protection of the safety and health of workers against risks resulting from exposure to biological agents during work, providing a high level of safety;

- To report annually the activity carried out according to the model available on the APA, I. P. website, including the conclusions of the audit carried out, if it has occurred.

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2.4 PHYSICAL RISK

2.4.1 ELECTRICAL RISKS

The most important electrical hazards are as follows:

- Electric shock. 60 volt is usually considered to be the safe voltage limit for the user. This limit assumes the distribution of current in the trunk in order to ensure that the current in the heart area has no chance of disturbing the heart's function. This risk increases with increasing electrical voltage available. The harmful effects of electric shocks increase and diversify as the voltage increases;

- Fire and burns, due to heating equipment and higher currents in socket contacts and components;

- Electrostatic discharges. Very important when working in environments with low humidity. When involving people, these discharges can cause sudden unreflective movements, which can result in accidents by dropping equipment, hitting other people, etc. They can also damage electronic equipment due to the hundreds of thousands of volts generated and, in suitable atmospheres, cause explosions or fires.

2.4.1.1 Safety rules

Risk of electric shock

To minimize the likelihood of electric shock, the following points should always be observed:

- A visual inspection of the equipment connections and their power cables should be made. Electrical plugs must be firmly inserted into the socket, and cables that show abrasion or cuts should be replaced immediately.

- All conductive elements in the area where electrical equipment is present (e.g. metal tables, equipment boxes, work surfaces, etc.) must be connected to ground/earth.

- When the voltage used in the laboratory is much higher than the main voltage (a limit of 500 V may apply in a simplistic way), stricter safety rules must be observed. The rules to be adopted must be defined by the Laboratory Safety Officer (RSL).

Risk of burns and fire

The risk of burns, and fire, with electric currents comes from localized heating by the Joule effect.

It cannot be stressed enough the problems of hotplates that are left on, "forgotten", when you finish using them. One safety rule is to assume that these devices are always hot! In long uses of this equipment it is absolutely essential to ensure that there are no combustible substances in the vicinity, since hot devices also heat the surrounding volume.

Heating can occur in the electrical connection contacts of equipment that has high currents (a 12 V source can cause a fire if it is passing a high current through a contact with a resistance of a fraction of an ohm, if it does not have sufficient heat dissipation). In classroom laboratories, there is the hazard of bad dimensioning of resistors in electric circuits, possibly leading to resistors reaching incandescence in a fraction of a second (a typical 10 ohm resistor with 5 volts applied will burn because it cannot dissipate the power, but if a resistor with appropriate dissipation is chosen it will work for years without any problem).

Excessive heating may also occur when connecting sockets make poor electrical contact. This is particularly important in equipment that has a high consumption. Visual checking of the contacts before plugging in is very important. The observation of oxidation, charring, etc., on the pins, even slightly, is a sign that the connections may be in need of checking (you cannot inspect the female contacts, but there will always be some transfer to the pins).

The use of extension cords with multiple sockets makes the current in the original socket the sum of the currents used in each equipment and enhances heating. This is the main reason why its use is considered a safety risk. Extensions should only be used in specific situations of short duration. In cases where it is necessary to use extension cords, the maximum current they support should be checked (many extension cords are designed for maximum currents lower than FCUP's fixed sockets).

Another important point in long extensions is that they should only be used completely unwound when used with currents close to the maximum value (if the wire is wound, the dissipation of the heat generated in the wires by the passage of the current is poor and the wire can heat up quite a lot). This heating can degrade the cable's insulation and lead to problems later on (e.g. cracks in the insulation that can cause the insulation to break down in certain circumstances)).

Another situation that can occur frequently is the breakage of some conductor wires, due to metal fatigue from successive bends of the cables at the same points. This increases the resistance locally, due to the decrease in the straight section of the conductor, increasing the temperature of the wire at that point. This problem is accentuated when there is a complete breakage of the conductor, leading to intermittent contacts with the possibility of arcing (this problem is recurrent in the power wires of laptops due to the tight winding of the wires for transport).

Electrostatic discharges

Electrostatic discharges in Porto are infrequent, due to the high atmospheric humidity, but they may be important in spaces with a controlled environment (laboratories with low humidity, or with negative temperature). In laboratories where these discharges may cause problems, measures should be taken to prevent them. First of all, all conductive surfaces must be connected to the ground of the building. It must then be ensured that the loads created by friction on the users are discharged. To this end, it must be ensured that the floor is moderately conductive and that there is always a good unloading path for the user (conductive chairs and seats, non-insulating footwear and clothing)

2.4.2 ELECTROMAGNETIC RADIATION

The main risks of electromagnetic radiation (excluding radio, X-ray and gamma radiation in this manual) are related to photochemical effects (ionizing radiation), thermal effects (burn and fire) and specific effects of laser short pulses.

Photochemical effects in the laboratory occur for ultraviolet wavelengths, and caution is only required for wavelengths above 180 nm (below, UV vacuum, air absorption is high, so there is only risk to humans inside a vacuum chamber). Exposure can be considered cumulative, and there is a risk of developing carcinomas. For very short pulses we can have photochemical effects due to multiphoton absorption (e.g. a very short focused pulse of a laser at 1.064 µm can cause effects that would only occur at 266 nm by the simultaneous absorption of four photons).

The risks associated with thermal effects are typically divided between risks of injury to the retina and the rest of the body, and risk of fire. Because the relaxed eye focuses light on the retina, it can create a point where energy is enough to degenerate proteins in the retina, causing cell death in that area. Safety standards are much tighter in the window where the eye is transparent (180 to 1400 nm). Wavelengths longer than 1400 nm are commonly referred to as "Eye Safe" because the radiation is absorbed in the initial area of the eye and does not reach the retina. They can, however, produce corneal burns). The safety calculations are based on the energy passing through the relaxed pupil (7 mm in diameter) and is deposited in the retina. For the rest of the body, the standards are less demanding, as the radiation is not normally focused. In addition, the impact of a high energy beam on flammable substances can be a source of ignition and cause fire.

In the case of light short pulses, risk assessment is more complicated. For long pulses (greater than a microsecond) it can be said, in a simplified way, that the total energy is the key factor. For shorter pulses many other factors have to be considered.

2.4.2.1 Lasers - Classification and safety rules

Class 1

When the level of radiation entering the pupil of the eye is not likely to create damage, laser equipment is classified as class 1 and no special care is required. Lasers with wide beams, or high divergence, that meet the limits of class 1, but with the use of equipment that concentrates light, such as binoculars, or microscopes, can exceed this limit, and are thus classified as class 1M.

The upper limits that define this safety class depend on the wavelength and duration of the pulses. In the figure below you can see the complexity of this calculation.

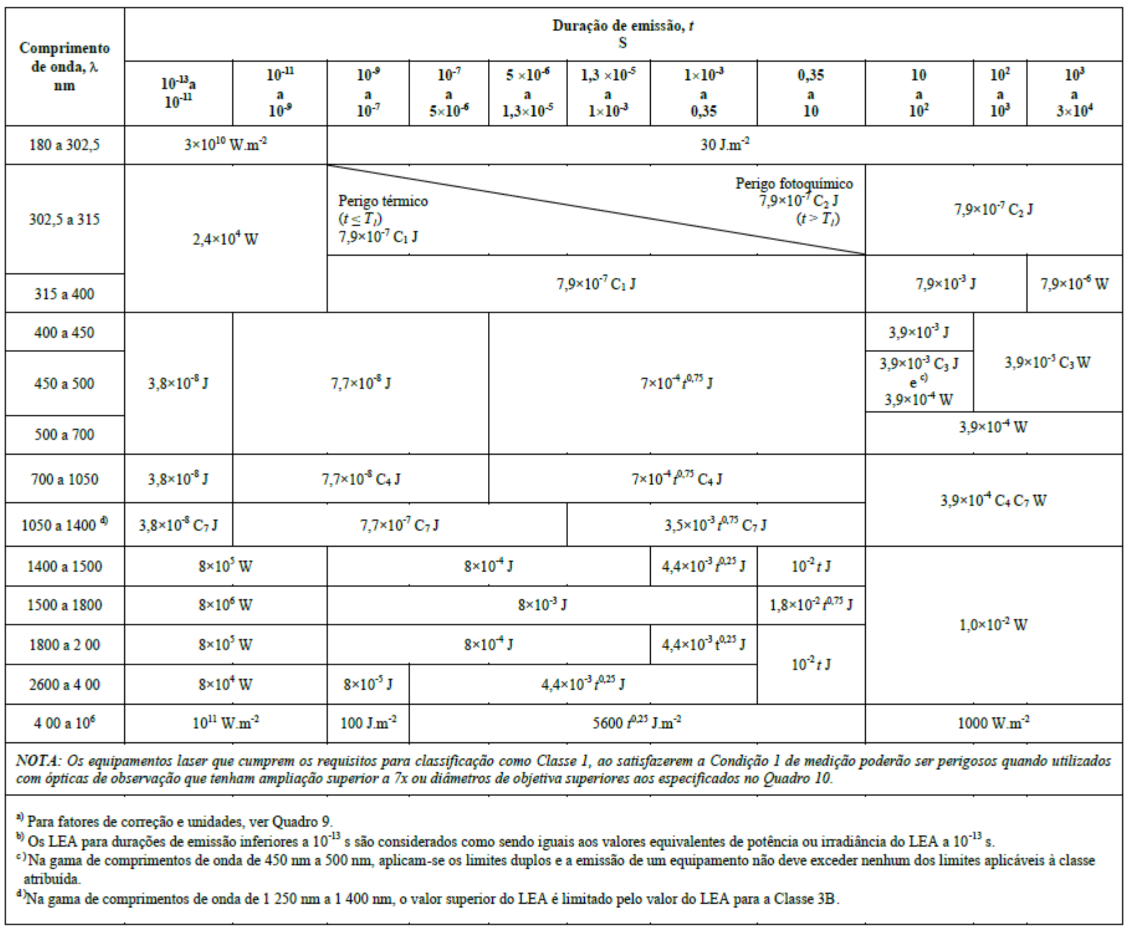
Some approximate examples for continuous lasers:

λ<400 nm (near UV) - < 8 µW or 10 µW/cm^2

400<λ<700 nm (visible) - < 0,4 mW or 0,52 mW/cm^2

700<λ<1150 nm (near IR) - < from 0,4 mW to 3 mW

Quadro 3 – Limites de emissão acessível para equipamentos laser de Classe 1 e Classe 1M e C_6= 1[a),b)]



Comprimento de onda, λ nm	Duração de emissão, t S										
	10^{-13} a 10^{-11}	10^{-11} a 10^{-9}	10^{-9} a 10^{-7}	10^{-7} a 5×10^{-6}	5×10^{-6} a $1,3\times10^{-5}$	$1,3\times10^{-5}$ a 1×10^{-3}	1×10^{-3} a 0,35	0,35 a 10	10 a 10^2	10^2 a 10^3	10^3 a 3×10^4
180 a 302,5	3×10^{10} W.m^{-2}		30 J.m^{-2}								
302,5 a 315	$2,4\times10^4$ W		Perigo térmico ($t \leq T_i$) $7,9\times10^{-7}$ C_1 J Perigo fotoquímico $7,9\times10^{-7}$ C_2 J ($t > T_i$)						$7,9\times10^{-7}$ C_2 J		
315 a 400			$7,9\times10^{-7}$ C_1 J						$7,9\times10^{-3}$ J		$7,9\times10^{-6}$ W
400 a 450	$3,8\times10^{-8}$ J	$7,7\times10^{-8}$ J			7×10^{-4} $t^{0,75}$ J				$3,9\times10^{-3}$ J	$3,9\times10^{-5}$ C_3 W	
450 a 500									$3,9\times10^{-3}$ C_3 J e c) $3,9\times10^{-4}$ W		
500 a 700									$3,9\times10^{-4}$ W		
700 a 1050	$3,8\times10^{-8}$ J	$7,7\times10^{-8}$ C_4 J			7×10^{-4} $t^{0,75}$ C_4 J				$3,9\times10^{-4}$ C_4 C_7 W		
1050 a 1400 d)	$3,8\times10^{-8}$ C_7 J	$7,7\times10^{-7}$ C_7 J				$3,5\times10^{-3}$ $t^{0,75}$ C_7 J					
1400 a 1500	8×10^5 W		8×10^{-4} J				$4,4\times10^{-3}$ $t^{0,25}$ J	10^{-2} t J	$1,0\times10^{-2}$ W		
1500 a 1800	8×10^6 W		8×10^{-3} J					$1,8\times10^{-2}$ $t^{0,75}$ J			
1800 a 2 00	8×10^5 W		8×10^{-4} J				$4,4\times10^{-3}$ $t^{0,25}$ J	10^{-2} t J			
2600 a 4 00	8×10^4 W		8×10^{-5} J	$4,4\times10^{-3}$ $t^{0,25}$ J							
4 00 a 10^6	10^{11} W.m^{-2}		100 J.m^{-2}	5600 $t^{0,25}$ J.m^{-2}					1000 W.m^{-2}		

NOTA: Os equipamentos laser que cumprem os requisitos para classificação como Classe 1, ao satisfazerem a Condição 1 de medição poderão ser perigosos quando utilizados com ópticas de observação que tenham ampliação superior a 7x ou diâmetros de objetiva superiores aos especificados no Quadro 10.

a) Para fatores de correção e unidades, ver Quadro 9.

b) Os LEA para durações de emissão inferiores a 10^{-13} s são considerados como sendo iguais aos valores equivalentes de potência ou irradiância do LEA a 10^{-13} s.

c) Na gama de comprimentos de onda de 450 nm a 500 nm, aplicam-se os limites duplos e a emissão de um equipamento não deve exceder nenhum dos limites aplicáveis à classe atribuída.

d) Na gama de comprimentos de onda de 1 250 nm a 1 400 nm, o valor superior do LEA é limitado pelo valor do LEA para a Classe 3B.

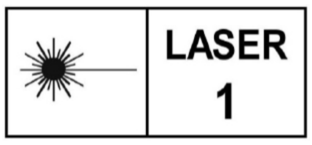
(source: Portuguese Standard NP EN 60825-1 (Safety of laser equipment. Part 1))

In terms of labelling, it is required that the equipment has an information label with a black border and yellow background (the size of the characters and the frame is specified in the NP EN-60825-1 standard), and that it is affixed where the exposure does not exceed class 1.

For class 1, the following labels shall be used:

EQUIPAMENTO LASER DE CLASSE 1

or alternatively



For class 1M the label should be as follows:

RADIAÇÃO LASER
NÃO EXPOR OS UTILIZADORES DE DISPOSITIVOS ÓPTICOS TELESCÓPICOS
EQUIPAMENTO LASER DE CLASSE 1M

or alternatively

Class 2

This grade is unique to visible lasers.

In class 2 one can work without eye protection as long as everyone present in the laboratory is properly trained and does not look directly at the beam (there is no danger in looking at the reflection on diffusing surfaces). Exposure of the retina for less than 0.25 s (maximum time to look away) is considered safe. As in class 1, if the beam is extensive, this laser may not be safe for those who use equipment that concentrates light (binoculars, telescopes, etc.), and this equipment is considered class 2M.

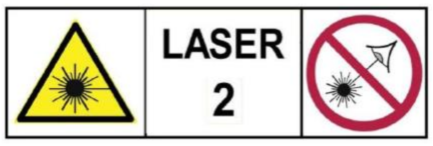
The upper limit of visible lasers in this class is 1 mW, or 1,3 mW/cm^2.

The use of class 1 and class 2 laser beams, although they do not cause permanent damage, can cause accidents to machine operators who may be dazzled by exposure to these beams (it is considered a crime to have a laser pointer directed at a road, or at a route used by aviation).

In class 2 the following labels shall be visible:

RADIAÇÃO LASER
NÃO OLHAR FIXAMENTE PARA O FEIXE
EQUIPAMENTO LASER DE CLASSE 2

or alternatively



In class 2M the following labels shall be visible:

RADIAÇÃO LASER
NÃO OLHAR FIXAMENTE PARA O FEIXE NEM EXPOR OS UTILIZADORES DE DISPOSITIVOS ÓPTICOS TELESCÓPICOS
EQUIPAMENTO LASER DE CLASSE 2M

or alternatively

Class 3R

Class 3R considers the limit of visible lasers at 5 mW, or 6,5 mW/cm^2.

Class 3 users must have specific safety training in order to use them.

It is recommended to wear eye protection equipment to avoid accidental incidences on the eyes during alignment procedures, and whenever reflective objects may be introduced into the path of the laser beam.

Procedures shall be used to prevent access by unauthorised persons.

The labelling to be used for class 3R is as follows:

RADIAÇÃO LASER
EVITAR EXPOSIÇÃO DIRETA DOS OLHOS
EQUIPAMENTO LASER CLASSE 3R

or alternatively

Classes 3B and 4

In these classes, protection is mandatory, even for experts.

The use of personal protective equipment (PPE) is mandatory, such as goggles that comply with the absorption standards for the wavelengths in use, and protective clothing.

The use of beam-blocking interlocks and standard signage on access doors is mandatory, as well as light signaling of the laser's operation on the outside.

It is important to note that in the laser safety assessment it is necessary to take into account all the spaces that the laser beam may accidentally reach (such as other nearby spaces through glass in the doors, other buildings through the windows, etc.).

The labelling to be used for class 3B is as follows:

AVISO - RADIAÇÃO LASER
EVITAR EXPOSIÇÃO AO FEIXE
EQUIPAMENTO LASER DE CLASSE 3B

or alternatively

The labelling to be used for class 4 is as follows:

PERIGO – RADIAÇÃO LASER
EVITAR EXPOSIÇÃO DOS OLHOS OU DA PELE À RADIAÇÃO DIRETA OU DIFUSA
EQUIPAMENTO LASER DE CLASSE 4

or alternatively

A label must also be attached to the outlet opening for class 3R, 3B and 4 laser radiation:

ABERTURA LASER

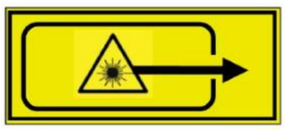
Or

ABERTURA PARA RADIAÇÃO LASER

Or

EVITAR EXPOSIÇÃO RADIAÇÃO LASER EMITIDA POR ESTA ABERTURA

or alternatively



2.4.2.2 Safety rules

There are different control rules depending on the laser category.

Category 1 lasers do not require any controls or safety measures.

The use of category 1M lasers requires some safety measures from the operator. The laser beam propagation should be delimited in such way that there is no possibility of it being observed by someone with binoculars or telescopes. This condition imposes a limitation on its use in open spaces (windows should also be considered). An example: an expanded beam to a section of 1 m^2 with 10 W of power (class 1M) is considered safe to be used in a room with children, as long as it is ensured that none have binoculars, or a simple magnifying glass, however it can be considered a very high risk if you have the possibility of exiting through a window (given the small divergence of the expanded beam, Their casual observation from miles away with binoculars would lead to permanent damage with an exposure of hundredths of a second).

The use of class 2 lasers means that no one should look directly into the beam (only prolonged exposure can cause permanent damage). These lasers require their users to have this knowledge and restrict access to the beam to persons who are informed and responsible (e.g. small children should be unable to approach the beam).

The use of category 2M lasers requires the operator to take the same precautions as those of class 1M in relation to observation with light concentrator systems by people near or far.

The use of class 3R lasers requires that the laser beam is as constrained as possible in order to prevent accidental exposures of the users' eyes. Users of this class of laser must have safety training in order to use them. It is recommended to wear eye protection equipment to avoid accidental incidences on the eyes in alignment procedures, and whenever reflective objects may be introduced into the path of the laser beam (the incidence of the beam on the skin is innocuous, but reflection on watches and jewelry can direct the beam into the eyes). Procedures should also be used to prevent access by unauthorised persons.

Class 3B and 4 lasers require safety training from users and visitors. The use of personal protective equipment (goggles that comply with the absorption standards for the wavelengths in use and protective clothing is mandatory). The use of interlocks that block the beam, and standardised signage on access doors is mandatory, as well as light signalling of the laser's operation on the outside.

It is important to note that in the safety assessment it is necessary to take into account all the spaces that the laser beam may accidentally reach (other spaces nearby through glass in the doors, other buildings through the windows, etc.).

2.4.3 Noise

Contact the CCSL (ccsl@fc.up.pt) to provide an analysis of noise exposure situation, If any of the following occurs:

(1) noise in the workplace usually or occasionally causes a nuisance, such as tinnitus or other;

(2) frequent voice elevation in conversations between people who are at a distance < 0.50 m;

(3) (1) or (2) and ototoxic substances (e.g. toluene, styrene, trichloroethylene, mercury, carbon disulfide are found);

.

All these situations are especially aggravated in case of pregnancy.

2.4.4 Cryogenic fluids

Handling of cryogenic liquids

Always handle these liquids with care to avoid skin burns.

Run the tasks slowly to minimize boiling and splashing. Always use tweezers to remove objects immersed in cryogenic liquid.

Never touch tubes or vessels (even non-metallic) without thermal insulation, containing cryogenic liquids. Fabrics stick to extremely cold materials

Cylinders and dewars should not be filled to more than 80 % capacity, as the expansion of gases during heating can cause overpressure.

Safe transportation

The presence of cryogenic liquids in confined spaces, such as elevators, can easily result in the formation of a suffocating atmosphere due to the displacement of oxygen, especially in the event of a stoppage between floors due to breakdown. Thus, the transport must be carried out without the presence of people inside the elevator, placing clearly visible signs warning that no one Is allowed to enter the elevator if it stops prior to the destination of the liquid.

Personal protective equipment (see more)

During the transfer and handling of cryogenic liquids the use of visors with safety goggles or chemical goggles is recommended.

Dry inflated gloves should be worn when handling objects that come into contact with cryogenic liquids and their vapours. Trousers must be worn up to the outside of the shoe, which must be closed.

Dry ice in transport and in cooling baths

Dry ice is widely used in the maintenance and transport of material that needs very low temperatures. You should not lower your head near open dry ice containers, as this can result in not only burns, but also suffocation from the accumulation of carbon dioxide. When dry ice is used in baths, it should be added slowly to the liquid part of the cooling bath to prevent foaming

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Liquid nitrogen cooled traps

Traps that open up into the atmosphere condense the air quickly. If the system is closed, the pressure can build up enough to break the glass equipment. Therefore, only sealed or evacuated equipment should use liquid nitrogen-cooled traps.

2.4.5 COMMON LABORATORY EQUIPMENT

2.4.5.1 General safety working practices

While each has its own specific safety requirements, some general guidelines for operating any laboratory equipment and instrumentation include:

- Wear the personal protective equipment (PPE) recommended by the manufacturer when using the equipment or instrument (hearing protection, face shield, etc.).
- Always keep the operating manual supplied by the manufacturer with the instrument.
- Follow the recommended maintenance procedures described in the manual.
- New users should be trained by qualified laboratory personnel and familiarize themselves with the operating manual, including all relevant safety information.
- Never remove hazard warning labels from an instrument.
- Make sure that all electrical equipment is grounded and properly connected.
- Call a certified technician to perform or supervise repairs.
- Disconnect the equipment from the power supply every time performing maintenance. In addition, discharge other energy sources that may coexist, such as batteries or accumulators.
- Do not modify any electrical components.
- Be aware of and trained in the specific hazards of your instrument. (i.e.: lasers, UV light, radiation sources, etc.)

2.4.5.2 Mixing & stirring equipment

Stirring and mixing devices commonly found in laboratories include stirring motors, magnetic stirrers, small pumps for fluids, and rotary evaporators for solvent removal.

These devices are typically used in laboratory operations carried out in a fume hood, and need to be operated in such a way that they do not generate electrical sparks, especially when working with flammable products (it should be confirmed from the manual if the equipment has any limitations in this regard).

Consideration should also be given to the consequences of possible agitator failure, electrical overload, or blockage of agitation impeller movement, as this type of equipment is often operated for long periods without supervision.

2.4.5.3 Heating equipment

Most laboratories use heating devices such as ovens, heating plates, heating blankets, oil baths, salt baths, sand baths, air baths, hot air guns, and microwave ovens.

Ovens

Do not dry glass material or any chemical sample containing flammable materials unless the furnace is explosion-proof. In the case of glassware, it should be rinsed with distilled water to remove the flammable solvent before placing it in the oven.

Ovens should not be used to dry any chemical sample that may pose a hazard due to its toxicity unless the oven is continuously ventilated to the outside of the laboratory

Heating plates

Older plates (without electrical spark protection) cannot be used for flammable materials.

The thermostats should be checked for corrosion, and repaired or replaced if this is the case

Heating blankets

Never exceed the manufacturer's recommended input voltage.

If the blanket has an outer metal housing that provides physical protection against damage by fiberglass, the outer metal housing must be grounded.

Older equipment may have asbestos insulation instead of fiberglass. If one of these pieces of equipment is identified, contact the departmental waste management officers to arrange its disposal.

IN THE EVENT OF LIQUID SPILLAGE: immediately disconnect the power supply and do not use again until it has dried properly, either in the sun or in a suitable oven.

Oil, salt and sand baths

It is necessary to contain the heated oil in a robust container.

When using oil, salt, or sand baths, awareness should be made that spillage of water or volatile substances over the baths may result in these hot materials being projected over a wide area and cause serious injury.

To avoid the generation of fumes or oil ignition, hot oil baths should not be overheated. You should always monitor the temperature of the oil bath to ensure that it does not exceed its flash point.

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Oil baths that are left unattended should be equipped/purchased with thermal sensors that will shut off electrical power if the bath overheats.

It is important to homogenize the oil baths well to ensure that there are no hot spots around the elements that heat the surrounding oil to unacceptable temperatures.

Mounting of the baths should be done carefully on a stable horizontal support, such as a laboratory platform that can be lifted or rested without danger of the bath tipping over. Iron rings are not acceptable supports for hot baths.

The equipment should be secured high enough above a hot bath so that in the event that the reaction begins to overheat, the bath can be lowered immediately and replaced with a cooling bath without having to readjust the equipment configuration.

Secondary containment should be provided in the event of a hot oil spill.

Heat-resistant gloves and safety goggles should be worn when handling a hot bath.

The reaction vessel used in a molten salt bath must be able to withstand very rapid heating to a temperature above the melting point of the salt.

Salt baths should be kept dry as they are hygroscopic, which can cause dangerous popping and splashing if the absorbed water vaporizes during heating.

Hot gas baths and tube furnaces

It is important to make sure that the heating element is completely closed.

For air baths constructed of glass, one must wrap the container with heat-resistant tape to contain the glass if it breaks.

When possible, it is advised to use sand baths instead of air baths.

For tube furnaces, one must carefully select the glass material, metal pipes, and gaskets to ensure that they are able to withstand the pressure.

Hot air guns

An extension cord should never be used to power a heat gun, as it may overheat and cause a fire or electric shock.

Never obstruct or cover the air inlet grilles, or operate the gun with the outlet nozzle directly against a surface.

You may not use a heat gun near flammable materials/atmospheres.

One should keep in mind the presence and direction of the heat produced.

Always switch off the tool before placing it on any surface.

Allow the tool to cool down before storing.

Never touch the hot metal mouthpiece with clothing or skin.

Never direct the airflow to the body. Do not insert anything into the nozzle of the gun.

Microwave oven

Microwave ovens specifically designed for laboratory use should generally be used.

To avoid microwave exposure, microwave ovens should never be operated with open doors.

The use of microwave ovens for laboratory use for food preparation is prohibited.

It should not be used to irradiate flammable materials or materials that emit toxic or corrosive vapours, except on intrinsically safe instruments.

Do not use metal containers or objects containing metal (e.g. magnetic stir bars) in the microwave.

Unopened containers should not be heated in the microwave oven. Even heating a container with a partially unscrewed lid poses a significant risk. Exceptions are made for instruments properly designed to support pressure vessels (e.g. microwave digesters). If the sterility of the contents is to be preserved, use cotton or foam tampons. Otherwise, cover the container with lab paper to reduce the potential for splashing.

It is not permitted to modify a microwave for experimental use.

Domestic microwave ovens may only be used for tasks comparable to domestic use (e.g. heating water). Its use in the dissolution/fusion of agar and agarose is acceptable, although a heating plate or bath should preferably be used. However, the following precautions should be observed for this specific situation:

- Do not use overfilled containers; an air chamber should be left to minimise the risk of the solution overflowing from the container (it should be taken into account that even outside the microwave, the hot solution may overflow from the still boiling container);
- Use the minimum power of the microwave and heat for short periods of time. Between heating periods, remove the container from the microwave and shake gently in a circular motion. This process should be repeated regularly and whenever the solution is starting to bubble;
- Constantly supervise the entire process;
- Wear heat-resistant gloves and safety glasses, due to the risk of explosion from non-uniform heating (see PPE).

2.4.5.4 Ultrasonication equipment

Laboratory-operated ultrasonic equipment may cause hearing and other damage. Typically, immersion probes exceed safety thresholds, so these devices must operate inside a sound-absorbing material housing, to substantially reduce emissions (most of which are inaudible). Alternatively, it can be placed in a dedicated room or with access conditioned to the use of hearing PPE. Read more in Noise on how to proceed with an exposure analysis of your ultrasonic equipment.

Direct contact of the body with liquids or solids should be avoided while undergoing high-intensity ultrasound of the type used to promote chemical reactions

2.4.5.5 Centrifuges

Centrifuges should be properly installed and used in accordance with the manufacturer's recommendations, and instructions for safe operating speeds should be followed.

It is important that the load is balanced each time the centrifuge is used, and that the lid is closed while the rotor is moving.

The shut-off switch must be working properly so that you can turn off the equipment if the top is accidentally opened.

For flammable and/or hazardous materials, the centrifuge must be under negative pressure and connected to a suitable exhaust system.

2.4.5.6 Rotary evaporators

The glass components of the rotary evaporator should be made of Pyrex or similar glass.

Glass containers should be wrapped in a shield (i.e. tape, netting or plastic coating) to protect against glass splinters in the event of an implosion.

The increase in the rotational speed and the application of the vacuum to the container whose solvent will be evaporated should be gradual.

When not located in a fume hood or ventilated compartment, rotary evaporators may require explosion protection, spill containment, and localized ventilation. Please refer to the section below "Vacuum Systems" to understand the requirements for cold steam traps for rotary evaporator.

2.4.5.7 Vacuum systems

Whenever experiments/operations are conducted in a vacuum, they shall be carried out behind a splinter barrier in the event of an implosion of the vessel. Ocular PPE should be worn.

All glass containers must be strong enough to handle the pressure differential flawlessly.

If the appliance or glass lines are chipped or cracked, the container must be replaced immediately.

One should wrap the glass containers with tape, netting, or plastic coating and place the vacuum trap inside a rigid container.

If a stand-alone vacuum pump is used with volatile hazardous materials, it is important to capture the vapors so that they do not degrade the pump oil or pass through the pump and be emitted into the laboratory. The capture device can be a dry ice or liquid nitrogen cold trap, but the type of trap depends on the specific chemicals.

Self-contained vacuum pumps shall deliver the gases to a fume hood or other means of extraction in the building, and not to the laboratory, when they are being used to evacuate a system containing corrosive, toxic or flammable substances. It is also possible to flush or absorb the gases coming out of the pump.

Other practices to ensure safe conditions when working with vacuum systems include the following:

Pumps

- Make sure that mechanical pumps have belt guards in place;
- It must be ensured that service cords and switches are free from defects;
- A trap should always be used in vacuum lines to prevent liquids from being drawn into the pump, general vacuum line or water drain if the pump is a water jet diffuser;
- Vacuum pump oil contaminated with condensate must be replaced and properly disposed of. Used pump oil should be disposed of as hazardous waste;
- The placement of a basin under the pumps makes it possible to retain oil leaks;
- Pumps should not be operated near containers of chemicals or flammable materials;
- Pumps are not run in a confined space.

Glassware

- Use only thick-walled round-bottomed glass material or specifically designed for vacuum systems;
- Carefully inspect the glass material for vacuum before and after each use. Discard any glass that is chipped, scratched, broken, or weakened in any way.

Cold trap

- Locate the cold trap between the system and the vacuum pump;
- Make sure that the cold trap is of sufficient size and also cold enough to condense the vapors present in the system;
- Check the cold trap frequently for blockages.

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2.5 CHEMICAL RISK

2.5.1 HAZARD CLASSES OF CHEMICALS AND THEIR COMMUNICATION

Chemical risk is associated with the properties of chemicals, which serve as criteria for the establishment of a hazard classification system (Global Harmonized System, GHS), associated with a substance or mixture. Hazard classes should be communicated in a variety of ways, as established in regulations adopted both at European level or more broadly (GHS, CLP, REACH).

Pictograms. These are an effective, visual and fast way of transmitting the **type of associated hazard(s)**, and they should go along with the products from their origin and whenever they are transferred to other containers, even on a laboratory scale (see below a table with the pictograms currently in use and their possible associated hazards).

Signal word. Other element of the commercial label: **Danger** or **Warning** (some hazards do not require a signal word).

Hazard Statements. Identify the associated hazards on the label (e.g. "H230 - May react explosively, even in the absence of air").

Precautionary Statements. Identify prevention, response, storage or disposal measures on the label (e.g. "P231 - Handle in an inert gas atmosphere").

Safety Data Sheet (SDS). It is a relatively extensive document, for longer consultation, which should also accompany the product from manufacture to the end user. It contains sections on product identification, hazard identification, composition, emergency action measures, storage and handling, exposure control/personal protection, physical and chemical properties, stability and reactivity, toxicological and ecological information, disposal considerations, transport, regulation and other information. Extended safety data sheets also contain a description of typical product usage scenarios, as well as their risk assessment and management measures.

SDS must be present at all places where products are used/stored. For certain purposes, such as rapid consultation in an emergency situation, there may be more **summarized SDS**, gathering the most relevant information in that context, such as the identification of the product, hazards, measures to take in case of emergency, stability and reactivity.

2.5.2 ASSESSMENT: MOST IMPORTANT CHEMICAL RISKS IN THE LABORATORY

When assessing the magnitude of a risk, one must take into account the two intrinsic components of the risk: the likelihood of it materialising and the severity of the damage potentially caused. In the case of chemical risk, these two components are usually expressed as conditions of use and hazardousness of chemicals, respectively.

An initial simplified approach is usually carried out, with the aim of categorising the risk associated with different chemicals at different levels, allowing for the identification of management priorities. If necessary, a further detailed assessment may follow. The latter already requires complex methods for assessing exposure (e.g., environmental measurements), or the use of the event tree technique, for example, for estimating the probability of possible accident scenarios.

Thus, based on the information accumulated over a long time, it is possible to have an idea of which type of events are the most frequent and/or most serious, linked to chemical risk in the laboratory, which are described below.

Risk of fire due to the use of flammable, pyrophoric and similar products.

Ignitions caused during the use or storage of chemical products are relatively frequent, as evidenced by those already occurred at FCUP, and at other Portuguese university institutions (*Aveiro, Nova Lisboa, Minho, ICBAS, FCUL, just to mention a few examples of fires reported in the media*). In addition to being frequent, they have an enormous potential for damage, and in extreme cases can destroy entire buildings, cause deaths and significant environmental damage.

Users of any FCUP laboratory where this risk exists will have to previously carry out the training described in the Training Plan and comply with what is stipulated in Fire prevention - Chemical products.

Accident with substances of acute toxicity to health and the environment.

According to the GHS, acute toxicity is that which is induced after a single dose or exposure to a substance or mixture (or multiple doses within 24 hours), or inhalation exposure (4 hours), and is usually associated with serious effects such as death or brain damage. In other words, accidental ingestion of a small dose, inhalation at a relatively low concentration, or relatively brief contact with the skin can be fatal. A massive release can reach catastrophic levels of damage in an area that extends beyond the FCUP itself. This can occur, for example, following a major fire affecting a storage site for such products.

Due to the high level of risk they pose, their acquisition, use and storage must be governed by what is stipulated in Acquisition, handling and storage of particularly hazardous substances: acute toxicity, carcinogenicity, mutagenicity and reprotoxicity (TACMR).

Serious harm to health from exposure to carcinogens, mutagens and reprotoxicants (toxic to reproductive function) (CMR)

Exposure to CMR is currently the greatest concern in terms of Occupational Hygiene. In fact, in Europe alone, each year, tens of thousands of deaths are attributed to work-related cancer. Unlike an accident that causes immediate damage, the effects of exposure to CMR manifest themselves in the medium/long term, what explains the tendency to underestimate these risks. This tendency must be tackled effectively.

Due to the high level of risk they pose, their acquisition, use and storage must be governed by what is stipulated in Acquisition, handling and storage of particularly hazardous substances: acute toxicity, carcinogenicity, mutagenicity and reprotoxicity (TACMR).

Serious injuries: eye, skin and others.

An explosive chemical, when subjected to heat, impact, friction, electrical discharge, etc., can produce a sudden and rapid release of gas and heat. When detonated under uncontrolled or unexpected circumstances, it can result in serious bodily harm or extensive property damage. Certain chemical reactions between two or more products can also result in explosions.

Due to the high level of risk they represent, the acquisition, use and storage of these products must be governed by what is stipulated in Acquisition, handling and storage of potentially explosive compounds.

The explosion of equipment containing chemicals under pressure or vacuum (e.g. in compressed gas cylinders or vacuum distillation) is another type of accident that is relatively frequent. Standards for minimising these risks should be found in **Compressed gases** and **Handling and storage of corrosive substances**.

Accidents without explosion, but involving projection or run-off of materials into the eyes and skin are frequent and can have very serious consequences, especially if they are materials with corrosive properties (GHS: cause irreversible damage under certain conditions). See **Personal Protective Equipment**.

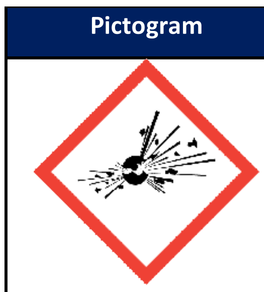
Damage to health from exposure to other hazard classes.

Products with sensitizing (allergenic) properties, specific target organ toxicity, irritating or narcotic properties (drowsiness, dizziness) are also considered hazardous, although generally associated with lower risk levels.

However, those that are in the form of gases, aerosols or are volatile, therefore easily disseminated in the environment, should be used under localized extraction.

The general measures of Basic Chemical Hygiene are always applicable.

Summary table of pictograms and their meaning (<u>these pictograms mean one or more of the following hazard characteristics</u>):

Pictogram	Meaning	Hazard characteristics
	**Explosive**	• Explosive substance • Self-reactive substance • Organic peroxide
	**Flammable**	• Flammable gas, aerosol, liquid or solid • Self-heating substance/mixture • Pyrophoric liquid/solid, may ignite on contact with air • Substance/mixture which, in contact with water, emits flammable gases • Self-reactive substance or organic peroxide, may cause fires under the action of heat
	**Oxidizing**	• Oxidant gas, solid or liquid, can cause or intensify fires and explosions
	**Gas under pressure**	• Gas under pressure, risk of explosion under the action of heat • Chilled gas, may cause burns or cryogenic injuries • Dissolved gas
	**Corrosive**	• Corrosive substance, can cause severe skin burns and eye injuries • Corrosive substance for metals

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	**Toxic**	• A substance that is highly toxic in contact with the skin, and can be fatal if inhaled or ingested
	**Irritant or harmful**	• Toxic (harmful) • Causes skin sensitization and skin and eye irritation • Irritating to the respiratory tract • Narcotic, causes drowsiness or dizziness • Hazardous to the ozone layer
	**Serious health hazards**	• Carcinogenic • Affects fertility and the unborn child • Causes mutations • Respiratory sensitizer • Toxic to specific organs • Aspiration hazard, can be fatal or harmful by ingestion or penetration into the respiratory tract
	**Hazards to the environment**	• Hazardous substance to the environment • Causes aquatic toxicity

2.5.3 MINIMIZATION OF CHEMICAL RISK

2.5.3.1 General preventive measures

Basic Chemical Hygiene

The measures described in this section complement the rules described in General Safety Rules.

Any spillage of reagents on the workbench or floor should be cleaned up immediately, always taking into account the nature of the spilled product (see more information under Emergency Procedures).

The workbench should always be clean. It should be cleaned after each phase of an experiment. Material containing hazardous chemical reagents must be decontaminated by the appropriate process before it is finally washed.

When handling a bottle of reagent, the outer walls should be cleaned immediately if any product runs down.

If the release of hazardous gases, vapours or aerosols is expected to occur, the experiment must be conducted under localised extraction, typically in an exhaust chamber (fume hood) (see Laboratory ventilation for more information).

Chemical hazard information elements

Inventory and Safety Data Sheets

Each laboratory or group of laboratories should have and keep up to date an inventory of chemical products, so that stocks can be monitored, unnecessary purchases may be avoided, and the accumulation of obsolete or unnecessary chemicals may be prevented. The inventory may be in physical or digital format and must be accessible to those responsible for Health and Safety at Work at FCUP, including departmental committees, and emergency services.

There should be a repository of the complete safety data sheets of the reagents and products (where applicable) used, produced or stored. This repository may be dematerialized (digital format) if there is another one in physical format containing the summary SDS with at least the following information: identification of the product, hazards, measures to take in case of emergency, stability and reactivity.

Labelling

Unambiguous identification of the chemical content is of utmost importance. It is very difficult to deal with unknown substances that pose unidentified dangers. Laboratory users are responsible for ensuring that chemicals and their associated hazards can be identified throughout their lifetime.

- Original commercial containers: The labels of the original containers should not be removed or disfigured until the container is empty and rinsed. No chemical should be accepted without a suitable identification label. Original containers must be labeled with the received date and the date of opening. This is particularly important for peroxide-forming compounds and other chemicals that become unstable over time, and is good laboratory practice for ALL chemicals.

- Secondary containers: If the chemicals are removed from their original container and placed in a different container, this is referred to as a secondary container. The secondary container may hold the original chemical, a mixture of chemicals, or a dilution of a chemical in water or solvent.

Secondary containers must be labelled with:

- Full name of the substance(s). Abbreviations cannot be used, as these may be ambiguous;

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- Identification of the type of hazards by means of pictograms and hazard statements;
- Date of transfer or preparation;
- Name of the person who transferred or prepared the solution;
- If the container contains a solution or mixture, include concentrations and solvents used.

It is important to make sure that the label remains readable, and to use a permanent marker that does not dissolve in the water or solvent used, or to attach an additional adhesive label. The label should be checked periodically and replaced if it becomes unreadable. Containers stored in the refrigerator are particularly prone to losing their labels, so you should check them more often or place them in a second container with an additional label inside.

Very small containers can be labeled with a number or other code, as long as they are accompanied by a sheet that contains the necessary information.

- Temporary and transfer containers (used only while conducting an experiment). At the very least, they should be labeled with the name of the chemical. Common abbreviations whose meaning is clear to everyone in the lab, such as ACN in a chromatography lab, or PBS buffer in a biology lab, are acceptable. If an experiment continues overnight or for several days, the label should also contain the user's name.
- Containers that hold non-hazardous substances, such as water, should also be labeled to avoid confusion with other chemicals.

Prior preparation of the experimental work

Before starting any experimental work, the operator must ensure that he is informed of all the potential hazards of the reagents, intermediates and final products (consultation of labels and safety data sheets or other sources), as well as the equipment and techniques to be used. He must also verify that the appropriate conditions exist for the collection of the waste produced, complying with the procedure rules set out in **Waste management.**

Organisational measures

Stock management of chemical products aimed at minimizing the quantities existing in the laboratory, especially those of greater hazardousness, whose acquisition, storage, handling and disposal are subject to special standards.

Reduction of exposed persons to a minimum by limiting access to certain work areas with more hazardous substances. It may involve the separation of zones to carry out certain operations.

Reduction of exposure duration and intensity to a minimum.

Adaptation of dining areas outside the laboratory area.

2.5.3.2 Purchase of highly hazardous chemicals

Applicable to strong oxidizers, pyrophoric, water-reactive, explosives, peroxide precursors, acutely toxic, CMR.

Both the lab inventory and other potential users' inventory should be consulted beforehand to confirm the real need to make purchases.

The quantities purchased must be the minimum necessary to carry out the planned work. **Accumulation approaches with the arguments of possible future needs or economic advantage by purchasing in quantity are not acceptable**.

The purchase of these products should not be made without ensuring the appropriate storage and usage conditions are guaranteed (see the respective specific sections of this manual).

Hazardous chemicals should preferably be supplied in drop-resistant plastic or metal lined packaging (where available) rather than uncoated glass packaging.

Reception of chemicals from outside the Campus

All incoming shipments should be inspected to ensure that proper labels are present, and that containers are intact and in good condition. Any containers that leak should be placed in an appropriate secondary container and treated as a chemical spill.

If a container appears to be deformed or pressurized, the package should be insulated if possible and the container should not be opened. Activate the safety team immediately (see **Emergency Procedures**).

2.5.3.3 Storage of chemicals in the laboratory

The existence of the minimum quantities in the laboratory, in addition to limiting the potential for risk, also facilitates their storage in the laboratory.

Segregation by hazard classes

Reagent bottles should be arranged by hazard classes and not alphabetically (except within the same class), and those that are incompatible should be stored, if possible, in separate cabinets or racks, separated by sufficiently wide aisles (~ 1 m). If the quantity of reactants does not justify it or space does not allow it, the incompatible substance families may be stored in the same place, provided that they are separated by inert substances.

It should be noted that this is a simplified system, and that in some cases compounds of the same class may be incompatible (see SDS of each reagent). **See also the list of common incompatible compounds**.

There are several possible ways to organize products by classes. As an example, a simplified system adapted from New York University's MSL is presented:

i) Store solid reagents, liquid reagents (or solutions) and compressed gases in separate areas.

ii) Within each of these physical forms they are segregated into hazard classes.

- Segregate solid reagents as follows:
  - Oxidizing solids (oxidants)
  - Flammable solids
  - Solids reactive with water
  - All other solids
- Segregate reagents and liquid solutions as follows:
  - Inorganic acids
  - Organic acids
  - Corrosive liquids
  - Oxidizing liquids (oxidants)
  - Perchloric acid solutions
  - Flammable liquids
  - All other liquids
- Segregate compressed gases as follows (see more on **Compressed Gas Safety**):
  - Toxic gases
  - Flammable gases
  - Oxidizing and inert gases

iii) Reagents belonging to several of the hazard classes simultaneously should be included in the class considered to have the most serious damage potential, which may still be difficult to discern (in case of doubt, consult the departmental SC).

For corrosive, highly toxic, volatile or foul-smelling products, it is recommended to store them in a ventilated place, for example a cabinet (properly identified), with extraction to the outside of the building or to suitable filters. Fume hoods may not be used as a ventilated storage place (see **Laboratory ventilation**).

Desiccators

Desiccators should be used for chemicals that react with air or water or are hygroscopic. For example, air-sensitive chemicals can be stored in desiccators that can be vacuumed and/or filled with an inert gas.

It is necessary to make sure that different desiccators are used to separate incompatible chemicals.

Containers

Chemicals should be stored in containers made of compatible materials. For example:

- Some chlorinated solvents degrade plastics;
- Hydroxide solutions dissolve aluminum and wear down glass;
- Hydrofluoric acid dissolves glass.

Containers should be in good condition (with no signs of cracking or corrosion) and should have lids that can be closed tightly to prevent evaporation and/or leakage, even if the container is dropped.

It is important to regularly check for old chemicals and unneeded chemicals. Old bottles whose contents are likely to be degraded should be discarded. This will save storage space and remove unnecessary risks from the lab.

Arrangement of products on shelves or other supports

Reserve adequate storage space for chemicals within the laboratory.

Avoid storing materials on top of cabinets.

Chemicals should be easily accessible and, to reduce the risk of accident, should not be stored on shelves higher than 2 m in height.

Ensure that the weight of the containers does not exceed the load rating of the shelves (where known). Heavier items and larger containers should be stored on lower shelves.

Corrosive liquids and particularly hazardous substances should be stored below eye level.

Keep chemicals away from heat sources or direct sunlight. Use secondary containment when possible (use of basins, or bulkheads on shelves, for example). Substances that are incompatible with water should be placed away from water pipes/plumbing and out of reach of emergency sprinklers (if they are available).

Brackets should be made of metal (with ground wire) or other robust material, for most substances. For corrosive substances, metal is not suitable.

Wall-mounted shelves are not recommended for chemical storage. In case of absolute necessity, this should happen on sturdy walls with a good fixation.

(see also the specifics of the storage of **flammable**, **potentially explosive** and **acutely toxic products or CMR**).

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2.5.3.4 Safe transport of chemicals

Containers:

- Hazardous chemicals must be transported in labelled closed containers that prevent leakage and do not present external contamination (e.g. run-off).

- If the container is breakable (glass, for example), it should be placed inside a suitable secondary container.

Transportation between labs, floors, and buildings on Campus:

- When possible, only freight elevators should be used when moving hazardous chemicals between floors.

- The use of trolleys is recommended. These should be stable under the load and have wheels with enough cushioning to overcome uneven surfaces without shaking or stopping suddenly.

In the case of transporting multiple hazardous chemicals:

- Chemicals should be grouped by hazard class compatibility and each group should be placed in a larger container while being transported.

- Containers used to transport multiple chemicals should be lined with an absorbent material to cushion the load. Multiple glass jars on the same tray should be cushioned using absorbent to prevent the bottles from bouncing against each other.

2.5.3.5 Fire Prevention – Chemicals

Flammable liquids

Vapors from flammable liquids can be ignited easily by an ignition source, such as a flame, a hot surface, a spark generated by electricity (static or otherwise), mechanical work, or heat released by a chemical reaction. The greatest risk is posed by solvents that are highly volatile and have a lower flammability limit (minimum concentration in the air to be able to ignite, LFL), as the vapor can reach ignition sources several meters away, still above LFL. If the vapor is ignited, the fire can quickly reach the bulk liquid. A flammable mixture of vapor and air at certain proportions can explode violently. The vapors are generally denser than air and will accumulate near the floor or bottom of the fume hood. Most flammable solvents damage the skin and are toxic by inhalation.

The following basic precautions must be followed when handling flammable liquids:

- They should only be handled in areas free of ignition sources. Never heat a flammable solvent with a Bunsen burner or other open flames. Flames should only be used after careful consideration of the equipment and experiments taking place in neighboring areas.

- Gas burners should never be operated unattended. Inspect the gas piping regularly and discard whenever it is found to be hardened or deteriorated.

- Ventilation is one of the most effective ways to prevent the formation of flammable mixtures. Any transfer, heating in open containers or any other handling of flammable substances, must be done with localized extraction (e.g. in fume hoods with adequate exhaustion).

- Keep all containers of flammable solvents tightly closed when not in use.

- Always wear laboratory PPE to avoid contact with the skin: closed-toe shoes, clothes that cover the legs, safety googles, gloves and lab coat. The lab coat must be flame retardant or at least 100 % cotton. Most synthetic materials melt quickly if ignited (see more **information on PPE**).

Transference of large quantities

By transferring large amounts of flammable liquids (> 5 L) to a smaller container, the flow of the liquid can create static electricity, which in turn can result in a spark. The build-up of static electricity is possible either by using a pump or by pouring the liquid manually.

Transfers of large quantities are likely to produce splashes or spills, and must be carried out in places with specific means to prevent such accidents and to ensure, if necessary, their collection and drainage to a safe place and in adequate ventilation conditions.

In these situations, consult SIMS and departmental SC to assess the best way to discharge static electricity safely and avoid splashing/spilling.

Storage in the laboratory

Solvents should be stored in the cabinets available exclusively for this purpose, preferably with fire resistance, in each laboratory. They should not be stored in the fume hood, cupboards under the fume hood, countertop, floor or shelves.

The storage limit of organic solvents in the laboratory will be the storage capacity of these cabinets.

All flammable storage cabinets must be clearly labeled with "Flammable – Keep Ignition Sources Away" or an equivalent message.

Flammable storage cabinets should never be located next to exit doors.

Flammable storage cabinets should not be used to store corrosives, which can corrode the cabinet, or other incompatible products (see more on **storage incompatibilities**).

Ventilation of flammable cabinets is not recommended as it reduces their fire protection effectiveness. If ventilation is required to prevent chemical exposure or to reduce odorous vapors, installation should be done by a qualified technician.

Refrigerator and Freezer Storage

Storage of flammable chemicals that require refrigeration or freezing should be done in a refrigerator or freezer that is designed for the safe storage of these types of substances.

Out-of-lab storage

Storage outside the laboratory, generally for larger quantities, is only allowed in places properly prepared for this purpose, and provided for in the fire risk plants submitted and approved within the scope of the Fire Safety in Buildings regulations.

Collection of waste flammable liquids

Flammable waste should be collected in the appropriate containers and clearly labelled as flammable for further disposal. The same handling precautions apply as for flammable liquids. See more information about Waste Management.

Reactive substances capable of generating or intensifying fires

The purchase of the compounds described in this section is subject to the rules set out in **Acquisition of Highly Hazardous Chemicals**.

Oxidizing agents

Oxidizers can initiate or promote the combustion of other materials. Oxidizing agents include inorganic halogenates, nitrates, chromates, persulfates, and peroxides. Several accidents occur due to oxidizer residues being dumped into common waste containers.

For safe handling, when working with strong oxidizers you should at least wear closed-toe shoes, long trousers, a lab coat, safety goggles with side shields and gloves.

When the procedure requires mixing a strong oxidant with an organic chemical, more specific information about the reactivity of these chemicals should be sought. Mixing can result in a violent reaction, so the oxidants should be used in a fume hood, with a protective barrier between the operator and the hazard.

Never return excess product to its original container. Contamination of the material can cause an unwanted and dangerous reaction.

Some common oxidants: ammonium dichromate, lithium and potassium perchlorates, ammonium, guanidine and strontium nitrates, nitric, sulphuric, chromic and perchloric acids, sodium and potassium permanganates, chlorine (liquid or gas), oxygen (liquid or gas).

Compounds that can polymerize

Polymerization can be dangerous when the reaction releases large amounts of energy, which can cause other substances to ignite.

Some common chemicals that can polymerize: acrylic acid, acrylonitrile, 1,3-butadiene, isopropenyl acetate, styrene, vinyl bromide, vinylpyridine.

Pyrophorics

The oxidation of these compounds by oxygen in the air proceeds so rapidly that ignition occurs spontaneously. Training in the handling of pyrophorics should include practical instruction given by someone experienced in the use of these chemicals. New users should only handle pyrophorics when they feel confident that they can perform the procedure safely. The use of a flame retardant lab coat, gloves and safety goggles is required.

Some common pyrophorics: barium metal, potassium metal, rubidium metal, magnesium powder, tert-butyl lithium, silane, triethylphosphine, methyl lithium, phosphorus.

Water-reactive chemicals

Alkali metals, many organometallic compounds, and some hydrides react with water to produce heat and hydrogen (flammable) gas. Some of these reactions occur violently, and extra care must be taken to handle and store these compounds away from washing stalls, water baths and other sources of steam or water aerosols.

Training for their handling should include practical instruction given by someone experienced in the use of these chemicals. New users should only handle water-reactive chemicals when they feel confident that they can perform the procedure safely. It is required to wear a lab coat, gloves and safety googles.

Common examples: sodium metal, potassium metal, antimony trichloride, phosphorus pentachloride, lithium tert-butyl, calcium hydride, titanium(IV) chloride, bromic acid, phosphorus pentasulfide, trimethylchlorosilane.

Transport of flammable liquids and reactive compounds

The transport of flammable liquids and reactive compounds that can cause or intensify fires must be carried out in accordance with the established in Safe Transport of Hazardous Chemicals.

Flammable gases

Flammable gases are also very common in the laboratory, especially hydrogen and acetylene. Their tanks should be located outside the building in suitable enclosures.

All standards described in Compressed Gases apply.

2.5.3.6 Acquisition, handling and storage of potentially explosive compounds

The purchase of these compounds is subject to the rules set out in **Acquisition of Highly Hazardous Chemicals**.

In the manuals of the laboratories where they are used or stored, the additional precautions taken in relation to these compounds should be documented, supplementing the instructions given below. Training for these tasks should include practical instruction given by someone with experience in the use of these chemicals.

Peroxide precursors

Some chemicals form peroxides over time. The two most serious hazards associated with peroxides are fires and explosions when exposed to heat, shock, or friction. The decomposition of peroxides can initiate explosive polymerization reactions. Peroxides can also oxidize human tissue, cotton, and other materials.

Peroxide precursors should be dated after opening and checked periodically. Safe handling, periodic testing, and removal of any detected peroxides should be described in the specific MSL (e.g., refer to the website of the Division of Research Safety at U. Illinois https://drs.illinois.edu/Page/SafetyLibrary/PeroxideFormingChemicals or to the U. Washington at www.ehs.washington.edu/system/files/resources/Peroxide_Forming_Chemicals.pdf).

Note on acquisition: Many known peroxide precursors can be purchased with oxidation inhibitors, such as butylated hydroxytoluene (BHT). If the inhibitor does not interfere with the use of the chemical, this option is strongly recommended. However, it should be noted that as the inhibitor is depleted over time, peroxides will naturally begin to form; It should also be noted that distillation processes will remove the inhibitor.

Note on labelling: An additional label containing the warning "Peroxide precursor" and the date of opening must be included, either on the original container or on a secondary container.

The following are the most common peroxide precursors, in 3 groups of different reactivity:

- **Group A**: serious risk after prolonged storage, especially after exposure to air. Chemicals in this group can form amounts of peroxides that can cause an explosion without the need of concentration. All of the chemicals listed below have already been responsible for fatalities:

  Main precursors: *butadiene*, isopropyl ether, sodium amide, chlorobutadiene*, potassium amide, tetrafluoroethylene*, divinyl acetylene, metallic potassium, vinylidene chloride (* when stored in liquid form)*.

- **Group B**: chemicals in this group form amounts of peroxides that do not normally cause an explosion but can pose an explosion hazard when concentrated by evaporation or distillation. Most are volatile enough that multiple openings of a single container can result in a significant and dangerous concentration of peroxide. They should be stored in a dark place, and tests for peroxide formation should be carried out every 12 months after opening. Also, always test before distillation or evaporation. If peroxides are detected, discard the chemical as hazardous waste or remove the peroxides.

  Main precursors: *acetals in general, dicyclopentadiene, 4-methyl-2-pentanol, acetaldehyde, diethylene glycoldimethyl ether, 2-pentanol, benzyl alcohol, diethyl ether, 4-penten-1-ol, 2-butanol, dioxane, 1- and 2-phenylethanol, chlorofluoroethylene, ethylene glycol ether acetates, cumene, furan, tetrahydrofuran, cyclohexene, 4-heptanol, tetrahydronephrene, cyclooctene, 2-hexanol, vinyl ethers, 2-cyclohexen-1-ol, methylacetylene, other secondary alcohols, cyclopentene, 3- methyl-1-butanol, isopropanol, decahydronaphthalin, methylcyclopentane, diacetylene, methylosobylketone*.

- **Group C**: the formation of peroxides can initiate explosive self-polymerization. In this group, the potential for peroxide formation is higher for liquids than for gases. It should be stored in a dark place and tested for peroxide formation every 6 months after opening and immediately before use. If peroxides are detected, discard the chemical as hazardous waste or remove the peroxides.

  Main precursors: *acrylic acid, methyl methacrylate, vinyl chloride, butadiene**, pestyrene, vinyldiene chloride, chloroprene**, tetrafluoroethylene**, vinylpyridine, chlorotrifluoroethylene, vinylacetylene, ethyl acrylate, vinyl acetate (** when stored as a gas).*

Nitrated compounds

Nitrated compounds, both organic and inorganic, constitute the largest class of compounds that are explosive when dehydrated.

When purchased (in the smallest amount possible), the container should be weighed and the initial mass recorded. Do not break the seal on the lid until the chemical is in active use. Before each subsequent use, weigh the container again and, if the mass has decreased, add an appropriate solvent (see SDS) to replace the lost mass. After the reagent is opened and an aliquot is removed, record the mass of the container again. It is important to visually inspect the container before each use and clean the neck, lid and threads with a wet cloth before resealing.

Common compounds: *diphenylhydrazine, nitrocellulose, 3-nitrotoluene, trinitrobenzene, trinitrophenol (picric acid), trinitrotoluene*.

Picric acid is usually purchased as a moistened mixture with 10% water, as it becomes highly unstable if dehydrated. Extreme heat, or electrical discharge can detonate this acid. When hydrated, picric acid is an orange, compact crystalline solid with the consistency of irregular aggregates of sand. When dried, picric acid is a crystalline solid with visible air pockets below the surface. Picric acid readily forms explosive metal picrates, which are extremely sensitive to shock and will detonate at the slightest movement or vibration. Do not allow picric acid to come into contact with readily oxidised metals, nor store in a container with a metal lid. Lead, iron, and copper are particularly dangerous.

Other potentially explosive compounds or mixtures (non-exhaustive list)

Aqua Regia: is a 3:1 mixture of hydrochloric acid and nitric acid, respectively. It is commonly used to remove noble metals such as gold, platinum and palladium from substrates, in the washing of glassware to remove trace organic compounds or in the digestion of samples for analysis, among other applications. It is extremely corrosive and can result in explosion or skin burns if not handled with extreme caution. Always use glass containers (preferably Pyrex). *Aqua regia* corrodes most metals and some plastics. Mix the solution in a fume hood with a protective barrier between the operator and the mixer. When preparing the *aqua regia* solution, always add the nitric acid to the hydrochloric acid slowly, and leave the hot solution in an open container until it cools. Always use a freshly prepared mixture and never store in a closed container. Oxidation over time forms toxic nitrosyl chloride, nitrogen dioxide and chlorine gases, which will cause pressure in the container and may cause an explosion. Mixing *aqua regia* with organic compounds can also cause an explosion.

Piranha Solution: is mainly used to remove organic residues from substrates. The traditional piranha solution is a 3:1 mixture of sulfuric acid and 30% hydrogen peroxide. The solution can be mixed before application or applied directly to the material by adding sulfuric acid followed by peroxide. Always use glass containers (Pyrex preferably). The solution can degrade plastic containers. Make sure all containers are properly labelled. Always mix the solution in the fume hood, placing a barrier between the operator and the mixture. Wear appropriate PPE, including: acid-resistant lab coat and/or apron with sleeve covers, gloves (butyl) and goggles with side guards. Always add hydrogen peroxide to sulfuric acid while stirring gently. DO NOT add sulfuric acid to hydrogen peroxide. The concentration of hydrogen peroxide must be kept below 30 % and must never exceed 50 %. Do not mix the piranha solution with incompatible materials, such as organic acids, organic solvents, or other organic materials, which may lead to an explosion. The piranha solution is intended for trace removal only. Ensure that all containers and substrates are rinsed and dried before coming into contact with piranha solutions. Never store the freshly prepared piranha solution in a closed or partially closed container. After use, allow the piranha solution to react overnight in a labelled container opened inside a fume hood before final disposal.

Ammonia reacts with iodine to form nitrogen triiodide, which is explosive. It also reacts with hypochlorite to yield chlorine. Mixtures of ammonia with organic halides react, sometimes violently, when heated under pressure.

Chlorine can react violently when in contact with hydrogen or hydrocarbons when exposed to sunlight.

Diazomethane and related compounds should be handled with extreme caution. They are very toxic compounds and their gases or solutions can explode spontaneously. According to this view, their ether solutions are safer.

Dimethyl sulfoxide breaks down violently when in contact with a variety of halogenated compounds. Explosions have also been reported when in contact with metal hydrides.

Explosions have been reported when ethylene oxide was heated in closed containers.

Halogenated compounds such as chloroform, carbon tetrachloride and other halogenated solvents should not be dried with sodium, potassium or other metals, as violent explosions usually occur under these conditions.

Hydrogen peroxide in concentrations above 30 % may break down violently when in the presence of iron, copper, chromium or other metals or their salts.

Lithium and aluminium hydride should not be used to dehydrate methyl esters or tetrahydrofuran as this very often causes fires. There are records that the reaction products of lithium and aluminium hydride with carbon dioxide are explosive.

Explosions have occurred due to high-pressure oxygen contacting with lubricating oil. Never use any lubricant on the connections to an oxygen cylinder.

Palladium or platinum in carbon, platinum oxide, Raney nickel and other catalysts must be filtered very carefully from the mixtures resulting from the catalytic hydrogenation reactions. The recovered catalyst is usually saturated with hydrogen, is very reactive and can spontaneously ignite when in contact with air. Another hazard involved in working with these catalysts occurs when adding more catalyst to a container that already contains hydrogen.

The use of perchlorates should be avoided whenever possible. They should not be used as dehydrating agents where there is a danger of contact with organic compounds, or in the presence of dehydrating acids of sufficient strength to concentrate perchloric acid to values above 70 %. Perchloric acid can be safely heated to temperatures in the order of 200 °C; However, contact of boiling acid or its vapour with organic matter, or even with easily oxidisable inorganic matter, will lead to violent explosions. Perchloric acid should never be brought into contact with oxidizable substances. Perchloric acid should always be used in a special fume hood (see Laboratory Ventilation). This should be cleaned weekly (including the vent pipe) to prevent spontaneous explosions from occurring.

Permanganates are explosive when in contact with sulphuric acid.

Barium, sodium and potassium peroxides, when in contact with combustible material, are easily ignited explosives.

Phosphorus (white and red) forms explosive mixtures when in contact with oxidizing agents. White phosphorus should be stored immersed in water, as it is spontaneously flammable when exposed to air. The reaction of phosphorus with aqueous hydroxides yields phosphine that can explode or ignite spontaneously when exposed to air.

Phosphorus trichloride reacts with water to give rise to phosphorous acid, which, under heating, breaks down into phosphine, which in turn can explode or catch fire. Great care should be taken when opening containers with phosphorus trichloride. The residues resulting from distillation under vacuum may explode when the system is brought to atmospheric pressure. These explosions can be avoided by using nitrogen for this purpose, or by letting the system cool down before bringing it to atmospheric pressure, or by doing so very slowly.

m-chloroperbenzoic acid should always be stored in appropriate plastic containers. When pure and stored in glass containers, it may explode.

2.5.3.7 Acquisition, handling and storage of particularly hazardous substances: acute toxicity, carcinogenicity, mutagenicity and reprotoxicity (TACMR)

Substances or mixtures that present acute toxicity (mainly **category 1 and 2**, **respiratory or dermal exposure routes**) can easily cause very serious damage such as death or brain injury, given the low doses or concentrations required to observe such effects.

Substances or mixtures with CMR properties deserve special attention in the legal regime for the promotion of safety and health at work (Law No. 3/2014 of 28 January), which dedicates the entire chapter V to the protection of genetic heritage, not only against chemical agents, but in general.

The high level of health risk posed by TACMR justifies the special control measures described below.

In the manuals of the laboratories where these compounds are used or stored, the additional precautions taken should be documented, supplementing the information given below. Training for the execution of the tasks should include practical instruction from someone with experience in the use of these chemicals. The purchase of these compounds is subject to the rules set out in Acquisition of Highly Hazardous Chemicals.

Approval procedure

Laboratory users who wish to use TACMR must first receive explicit written approval from the laboratory safety officer, who should only grant it if the user has received the appropriate training (documented in FFI). To do this, a form for approval of the use of particularly hazardous substances must be filled out. The information required on the form includes:

- Possibility of substitution for another product of lower risk;
- Identification of TACMR, physical characteristics and health risks;
- Consideration of exposure controls such as fume hood or glove boxes and personal protective equipment;
- Assignment of an area (fume hood, part of the laboratory, entire laboratory) specifically for experimental procedures with the substances involved;
- Storage and secondary containment plan, procedures for safe removal of contaminated waste, and decontamination procedures.

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Completed and approved forms should be kept in the laboratory records. The execution of the work shall continue under the conditions approved by the RSL.

The area allocated for this purpose must be marked with the following information:

Work area with high risk to health

ACCESS ONLY FOR AUTHORIZED PERSONNEL

Safe handling

The level of risk associated with TACMR requires stricter operational procedures in the laboratory, including:

Work habits

There must be no eating, drinking, chewing of gum, application of cosmetics, or storage of utensils, food containers, or food. All personnel must wash their hands and arms immediately upon completion of any procedure in which a TACMR product has been used and when leaving the laboratory.

Each procedure must be performed with the minimum amount of the substance, consistent with the requirements of the work. The use of a removable absorbent liner on work surfaces is recommended as it helps contain spilled materials and simplifies subsequent cleaning and disposal.

Ventilation / Insulation

Work must be carried out in a fume hood or with another form of ventilation/extraction if the chemical produces vapors, aerosols or is a gas. The linear velocity of the airflow in the fume hood (depends on the position of the sliding window) should be at least 0.4 m/s. Important: See more about the correct use of fume hoods in Laboratory Ventilation.

A glove box should be used if protection from atmospheric moisture or oxygen is required or when a fume hood does not provide adequate protection against exposure to the substance.

Highly toxic gases should be stored in a cabinet outside the building. See more in Compressed Gases.

Vacuum Lines

Vacuum lines should be protected with an absorbent or liquid trap to prevent TACMR compounds from entering the system. When using volatile TACMR compounds, a separate vacuum pump should be used. These procedures should be carried out inside a fume hood.

Personal Protective Equipment

The SDS of each specific product should be consulted for information on appropriate gloves, clothing, and respiratory protection. Laboratory clothing that protects everyday clothing, such as a fully fastened lab coat or a disposable coverall, should be worn but only in the laboratory area. When clothing decontamination methods are unknown or not applicable, disposable protective clothing should be worn. Disposable gloves should be discarded after each use and immediately after direct contact with TACMR compounds.

Storage, transport, decontamination and disposal

TACMR products should be stored in a limited access area. Additional storage precautions (i.e., refrigerator, extraction, flammable liquid storage cabinet, etc.) may be required for certain compounds based on other properties. Containers must be clearly labelled.

Double containment, such as the use of retention basins, should also be considered. Containers should be stored in trays or other containers made of polyethylene or other chemically resistant material. For its safe transport, the instructions presented here must be followed.

Contaminated materials must be decontaminated by procedures that decompose TACMR products to yield a safe product, or be removed for further disposal as hazardous waste. All work surfaces should be decontaminated at the end of the procedure or workday.

2.5.3.8 Handling and storage of corrosive substances

Corrosive chemicals are commonly found in laboratories as solids, liquids, or gases. These materials have the ability to damage tissues at the point of contact, being responsible for many serious skin and eye injuries. They can also cause considerable damage to the respiratory tract by inhalation.

Corrosive materials are most commonly strong acids and bases, but many other materials can be severely harmful to living tissues.

Skin contact with alkali metal hydroxides (e.g. sodium hydroxide and potassium hydroxide) is potentially more dangerous than with strong acids. Contact with alkali metal hydroxides usually causes deeper tissue damage because it provokes less pain than with acid exposure. The exposed person may therefore not wash the affected area sufficiently or not seek medical help promptly.

Handling of corrosives

Specific precautions to take when handling corrosive materials include:

- Do not work with corrosives unless an emergency shower and eyewash station are available within a distance of less than 10 seconds to travel;

- Make sure that acids are always added to the water and not the other way around;

- Be prepared for the release of heat when diluting or dissolving in water;

- Make sure that all work is conducted in a fume hood with adequate ventilation. If the fume hood does not have a retention basin or contains equipment or other materials Inside, use a plastic basin or tray to contain any spills and leaks;

- Always wear the appropriate gloves when working with corrosives. Neoprene and nitrile gloves are effective for most acids and bases. Polyvinyl chloride (PVC) is also effective for most acids. The lab coat typically used in laboratories (cotton/polyester) does not ensure full protection. A rubber-coated apron is a good complement to the lab coat. Safety googles should be worn, and if a projection is likely to occur, wear a face shield over the glasses. See more information about PPE.

Storage of corrosives

Corrosive liquids should not be stored above eye level. Store on a low shelf or inside a low cabinet.

Store acids in a plastic tray, a basin or a bucket, a tub or a rubber bucket to contain any spillage.

Acquire corrosives in plastic-lined containers, as this will reduce the risk if the container is dropped.

Store acids in purpose-designed cabinets or one that has a corrosion-resistant coating. Acids stored in ordinary metal cabinets will quickly corrode its interior. If an acid cabinet is not available, store in a plastic basin inside a wooden cabinet.

Nitric acid should always be stored away from other acids and organic materials, in a separate cabinet or compartment due to its high reactivity.

See additional information on chemical storage.

Handling and storage of hydrofluoric acid

Hydrofluoric acid can cause severe burns. Initial skin contact with hydrofluoric acid may not produce any symptoms, however, this acid can draw calcium from the skin and bones, resulting in serious injuries. Inhalation of hydrogen fluoride can be fatal.

Therefore, all the above precautions should be taken. Never store hydrofluoric acid in a glass container as it is incompatible. Hydrofluoric acid is usually purchased in a plastic bottle. Only the minimum amount necessary should be kept and stored separately in an acid storage cabinet.

Calcium gluconate creams are commercially available for the treatment of dermal exposure to hydrofluoric acid, which must be present at the sites of use/storage of hydrofluoric acid. Calcium gluconate reacts with hydrofluoric acid reducing the attack on calcium from the body.

2.5.3.9 Compressed gases

The following is an overview of the hazards associated with the handling and storage of compressed gases:

Asphyxiation: simple asphyxiation is the main risk associated with inert gases. Because inert gases are colorless and odorless, they can escape into the atmosphere undetected and rapidly reduce the concentration of oxygen below the level needed to support life. The use of oxygen monitoring equipment is strongly recommended for enclosed areas.

Fire and Explosion: these are the main hazards associated with flammable gases, oxygen and other oxidizing gases. Flammable gases can be ignited by static electricity or by a heat source such as a flame or a hot object. Oxygen and other oxidizing gases support the combustion of flammable materials. Increasing the concentration of an oxidizer accelerates the rate of combustion. Non-flammable materials under normal conditions can burn in an oxygen-enriched atmosphere.

Chemical Burns: corrosive gases can chemically attack various materials, including fire-resistant clothing. Corrosive gases can cause rapid destruction of skin and eye tissue.

Chemical poisoning: chemical poisoning is the main risk of toxic gases. Even at very small concentrations, a brief exposure to these gases can result in immediate or delayed serious injury.

High Pressure: all compressed gases are potentially dangerous due to the high pressure inside the cylinder. A sudden release of pressure can cause injury and property damage by the thrusting of the cylinder or the whipping effect of the pipes.

Cylinder Weight: a cylinder can weigh more than 60 kg. Moving a cylinder manually can lead to back or muscle injuries. Dropping or dragging a cylinder can also cause serious injuries.

The EN 1089-3:1997 Standard establishes a colour system for identifying the hazards associated with the contents of the cylinder. The main hazard colours in this European Standard are:

Yellow: Toxic and/or corrosive
Red: Flammable
Blue: Oxidant
Green: Inert

The most common gases have specific colors:

Acetylene - Maroon
Ammonia - Yellow
Carbon dioxide - Gray
Argon - Dark green
Air - Green
Nitrogen - Black
Oxygen – White
Hydrogen - Red
Helium - Brown

Equipment and piping assembly

The connection of equipment to a gas supply network, directly to a cylinder, or the installation of new supply lines, are critical operations that must be carried out by certified personnel. These should only be carried out after approval by the SIMS, which will verify, among other conditions, the non-violation of the emergency plan in force, namely the risk classifications of the spaces submitted to the national civil protection authority (ANPC).

Storage of compressed gas cylinders

All cylinders must be fixed to a wall or fixed bracket using a chain or strap placed at 2/3 of the cylinder height. The cylinders must be tied individually.

Oxidizing and flammable gases must be stored in areas separated by at least 6 m, or by a non-combustible wall.

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Cylinders must not be stored near heat sources. If storage is outdoors, the cylinders should be protected from weather extremes and wet soil to prevent corrosion. No part of a cylinder should be subjected to a temperature higher than 50 °C.

Cylinders may not be placed where they can become part of an electrical circuit.

The cylinder must be properly labelled as to its content.

The empty cylinders should be segregated from the full ones to avoid confusion. Still, treat empty cylinders as if they were full (don't forget that an empty cylinder is probably not completely empty).

Handling precautions

Cylinders should not be dragged or slid, but a suitable trolley equipped with a chain or belt should be used to secure the cylinder (see image below), even for short-distance transports. The cylinders must not collide with each other.

The cylinder cap should be left on until it is fixed against a wall or placed in a cylinder holder, and is ready for installation of the regulator. Cylinder caps protect the valve from damage in the event of a fall.

Pressure relief devices on valves or cylinders should never be altered.

Only wrenches or tools approved by the cylinder supplier should be used to remove a cylinder cover. Never use a screwdriver or pliers.

Position the cylinders so that the valve is always accessible. The valves are designed to be operated by hand, never with a wrench or other tool. Contact the supplier if this is not possible.

The cylinder valve should be kept closed except when in use.

When bubbling gas into a liquid, a suitable trap or check valve should be used to prevent the liquid from flowing back into the cylinder or regulator.

Pressure Regulator

The outlet connections of the cylinder valve are designed to prevent mixing of incompatible gases. Outlet connections vary in diameter and shape of tightening.

To set up and use the cylinder, you must follow these steps:

i) Connect the closed regulator to the cylinder. Never open the cylinder valve unless the regulator is completely closed. Regulators are specific to the gas involved. A regulator must be connected to a cylinder without straining the pipes. Make sure that the thread of the regulator and the cylinder valve are clean and not damaged. If the inlet of a regulator does not fit into the outlet of the cylinder, the socket should not be forced.

ii) Open the cylinder valve slowly until the high-pressure gauge registers the pressure in the cylinder. If the cylinder pressure reading is lower than expected, the cylinder valve may be leaking.

iii) If necessary, adjust the pressure control valve at the regulator outlet to the desired value.

iv) Check for leaks using a specific spray. Never use an open flame to detect leaks.

v) When you finish using the gas, close the cylinder valve and release the pressure from the regulator.

Leaking cylinders

Most gas leaks occur at the valve located at the top of the cylinder, and may involve the valve stem, valve outlet, or pressure relief devices. Laboratory personnel should not attempt to repair leaking cylinders.

If there is no risk of serious exposure of laboratory personnel, the cylinder should be moved to an insulated, well-ventilated area (away from combustible materials if the cylinder contains a flammable or oxidizing gas). Contact the safety post through the internal (#112) or external landline (see all numbers here).

Whenever a large or uncontrollable leak occurs, evacuate the area and immediately contact the safety post through the internal (#112) or external landline (see all numbers here).

Highly toxic gases

Highly toxic gases, such as arsine, boron trifluoride, diborane, ethylene oxide, fluorine, germanium, hydrogen cyanide, phosgene and silane, can pose a significant health risk in the event of a leak. The installation and use of these gases is subject to SIMS approval and to special precautions to be defined by the FCUP Directorate.

2.6 LABORATORY VENTILATION

General ventilation

In general, the FCUP laboratories work at negative pressure in relation to the corridor, due to the existing air extraction means. Keeping doors and windows closed, especially when there are fume hoods or equivalent, prevents hazardous or foul-smelling emissions from spreading through the hallway and the rest of the building.

There are also some positive pressure laboratories, as a way to protect them from the entry of untreated air from inside the building.

In either case, the supply and air outlet openings must never be blocked or covered. Any changes in the ventilation of the laboratory may compromise the safety characteristics of the room and the localized exhaust systems, such as fume hoods, biosafety chambers, etc.

Fume hoods and glove boxes

One of the main safety devices in a laboratory is the fume hood. A well-designed fume hood, when properly installed and maintained, can offer a substantial degree of protection to the user, provided it is used properly and its limitations are understood.

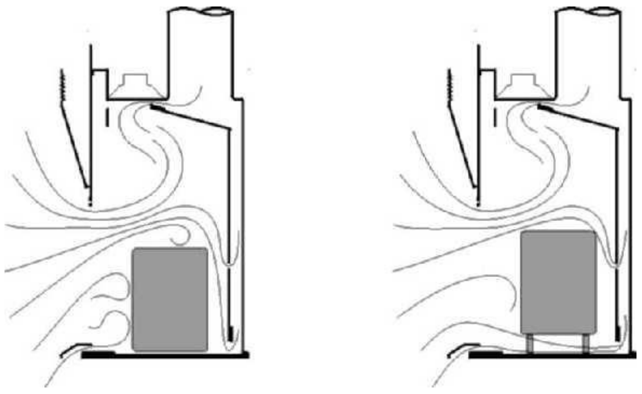
A fume hood is a piece of ventilation equipment that operates separately from the building's heating, ventilation, and air conditioning (HVAC) system. The main means of controlling chemical exposure by the respiratory route is the fume hood, so it should be used when working with toxic compounds or with a boiling point below 120 °C (some aqueous solutions may be an exception to this rule). You may need to use a closed system, such as a glove box or bag, for highly hazardous chemical materials (see more below).

Use of the fume hood

The instructions contained in the following points are sufficient for the correct use of the fume hood. However, it may be useful to complement it with an online training, such as the one from the Centers for Disease Control and Prevention (CDC, United States) available at *Fundamentals of Chemical Fume Hood Safety* | CDC.

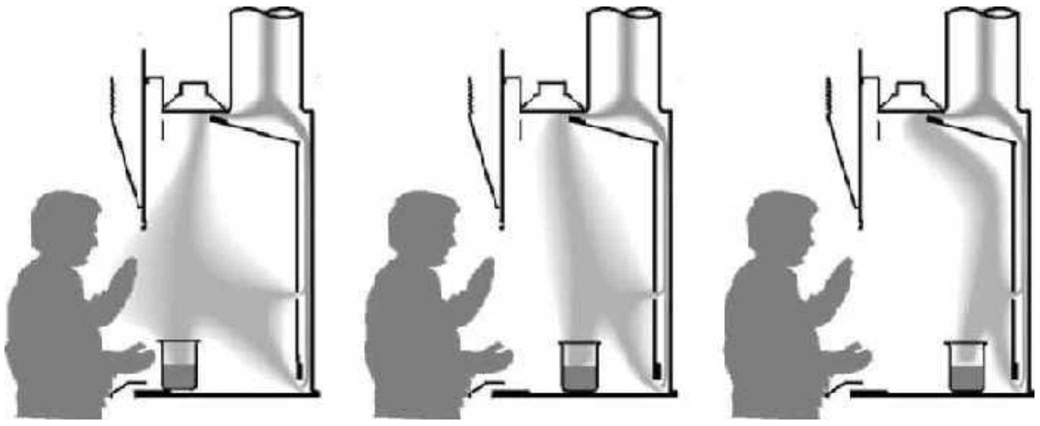
- Operability check – make sure the fume hood is in operation before starting to work. Some fume hoods have monitoring devices that indicate acceptable working conditions. Otherwise, a strip of low-density paper placed at the bottom of the protective strip or a small wind vane for low flows can be used as an airflow indicator. If there are any doubts as to the proper functioning of the fume hood, work should not be started and the RSL should be informed.

- Minimization of crossdrafts and swirls – Airflow to the fume hood is adversely affected by crossdrafts and swirls. Crossdrafts occur when people pass in front of the fume hood or when nearby windows or doors are open. Swirls occur around the user of the fume hood and around objects within it. To limit these effects, fume hoods should not contain unnecessary objects, and slits inside the fume hood that direct airflow should not be blocked.



Schematic representation of the effect of the presence of bulky objects inside fume hoods.

- The slit on the back of the work surface is essential for proper air movement. You should not place large pieces of equipment or a large number of bottles in front of the slit. The equipment should be placed in the back as much as practicable, leaving 15 cm distance from the rear. Work should be carried out at least 15 cm inside the fume hood opening to prevent crosscurrents and swirls from throwing contaminated air from inside the fume hood into the laboratory.



Effect of the position of contaminant sources within a fume hood.

- Sash: The sash should be kept as low as possible to improve the overall performance of the fume hood. The lower the sash, the better the protection against an unexpected chemical reaction. Procedures should be done with the sash at the level of the approved maximum height marking or lower (where present). When working with an open sash, use a separate safety shield such as a face shield.

- Solvent evaporation: the fume hood should not be used to dispose of chemicals by evaporation, except for evaporation of the residual waste film required for the decontamination and recycling of containers (see **Hazardous Waste Management**). Any procedure that emits large volumes of volatile chemicals should be equipped with condensers or traps to contain and collect hazardous vapor or dust.

- Storage: Do not store chemicals or other objects in the fume hood. Chemicals should be stored in proper cabinets.

- Flammable Liquid Vapour: Laboratory fume hoods are designed to reduce flammable vapours below lower flammability limits when properly operated and maintained. As an additional precaution, when a large volume of flammable vapor may be generated, use only spark-proof and explosion-proof electrical equipment (hot plates, stirring plates, centrifuges).

- Containers: All chemical containers inside the fume hood should be securely closed when not in use. One rule of thumb is that containers should be open for less than a minute – which is the maximum time it normally takes to transfer a small amount of chemical to another container and close both of them. All containers must be labelled as described in **General preventive measures**.

Fume hood performance testing

Departments, or their safety committees (SC), must request that SIMS carries out a functional performance test of a fume hood annually, or whenever malfunctioning is suspected. If a fume hood fails the test, it may be necessary to put it out of service until it is repaired by an outside company. In this case, SIMS or SC will notify the RSL, and an "OUT OF SERVICE" sign will be placed.

The test includes measuring face velocity and visualizing the effectiveness of restraint. Specific performance measures for each test are described below.

Face velocity: recording of values ≥ 0.3 m/s, with a sash height of not less than 45 cm. Containment test: use of dry ice or a non-toxic smoke generator for visual verification of containment effectiveness.

Fume hood for perchloric acid

Procedures using concentrated perchloric acid (> 70 %) or heating any amount or concentration of perchloric acid should be carried out in a closed system or inside a specially designed perchloric acid fume hood with flushing systems to prevent the accumulation of explosive perchlorates in the fume hood and ducts.

Glove boxes

Glove boxes should be thoroughly tested before each use and there should be a method of monitoring the integrity of the system (such as a pressure gauge). RSLs should develop instructions for use for each specific system in addition to this MSL.

Biological safety (CSB) and laminar flow chambers

There are two types of CSB. Class II Type A and Class II Type B1 units recirculate filtered air in the laboratory, so they are not permitted for chemical use. The Class II Type B2 unit is designed for the use of some chemicals, but is not a substitute for the fume hood. The use of chemicals in this type of chamber needs to be carefully evaluated so that the protective barrier (HEPA filters) is not destroyed by the products in question. The manufacturer's instructions on the use of chemicals must be strictly followed.

The handling of BRL-2 microorganisms should be done in Class II CSB.

CSB with UV lamps should contain appropriate signage.

Departments, or their safety committees (SC), must request that SIMS carries out an annual performance check, or whenever malfunctioning is suspected. If a CSB fails the test, it may be necessary to put it out of service until it is repaired by an outside company. In this case, SIMS or SC will notify RSL, and an "OUT OF SERVICE" sign will be placed.

Laminar flow chambers

Laminar flow chambers are designed to protect the work surface from contaminants and can blow into the operator's face. Therefore, any use of chemicals will cause the person to be exposed. Chemicals that are hazardous to health cannot be used.

Maintenance

All ventilation systems need periodic maintenance (clogged air inlets and outlets, loose belts, bearings that need lubrication, motors that need attention, etc.). Whenever any problem is detected, SIMS support should be requested via Infociências.

Before any maintenance of fume hoods or CSB, all internal surfaces must be decontaminated and/or cleaned. Maintenance may require access to storage cabinets that are located below or next to the fume hood/CSB. If access is required, all of this space and the surrounding area also need to be emptied, decontaminated, cleaned, and rinsed.

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2.7 Personal Protective Equipment

With regard to the subject of PPE, there are two European directives to be considered: Directive 89/656/EEC on the minimum safety and health requirements for the use by workers of PPE at work, and Directive 89/686/EEC on the approximation of the laws of the Member States relating to personal protective equipment, as amended in the meantime by Directives 93/68/EEC, 93/95/EEC and 96/58/EC, which are transposed into national law.

In order to be able to circulate freely on the European Union market, PPE must be CE marked and accompanied by a declaration of conformity issued by the manufacturer of the equipment or its authorised representative established in the European Union.

According to Decree-Law No. 348/93, PPE is all equipment, as well as any complement or accessory, intended to be used by the worker to protect himself from risks to his safety and health.

The use of PPE, as a risk control measure, should be implemented as a last resort, and priority should fall, in accordance with the General Principles of Prevention established in the legislation, on collective and organisational protection measures.

PPE must be appropriate to the risks to which the worker is exposed in the course of his work or in the course of his activity. It is, therefore, essential that workers have information and training that allows the correct use and maintenance of PPE.

There is a wide range of PPE available, but in the case of FCUP laboratories the use of **lab coats, goggles or visors, and gloves** are of particular importance.

Personal clothing and footwear

Personal clothing also contributes to the protection of the skin. Laboratory lesions are often found that could have been reduced in size if appropriate personal clothing was worn. It is recommended that laboratory personnel working with hazardous materials or organisms, or in their presence, have their skin covered from shoulders to hands and feet, by the combination of personal clothing and PPE.

Closed footwear should always be worn in the laboratory.

Lab coat

This PPE has several generic functions:

- Protective barrier – protects torso and arms from direct contact with hazardous substances; A good lab coat is at least semi-flame retardant, ideally 35% polyester/65% cotton. Polyester provides chemical resistance and ease of maintenance, while cotton provides flame resistance. It also protects personal clothing from dirt and contamination.

- Prevention of cross-contamination – keeping the lab coat in the lab traps any contamination within that lab and reduces or prevents cross-contamination in other areas.

- Quick removal - Can be removed quickly in an emergency; If a fire or a dangerous spill occurs, the lab coat (especially if it has quick-release buttons) can be removed in a matter of seconds.

In addition, the inclusion of an elastic wrist helps to keep the sleeves away from objects and thus maintain greater control of the workspace. It also protects the arms from dangerous drips that may occur.

Finally, a clean lab coat indicates professionalism and positively distinguishes a user from the laboratory.

The use of a tight (unopened) lab coat is mandatory when working with or in the proximity of hazardous chemicals, unsealed radioactive materials and biological agents.

The use of an especially flame-resistant lab coat (Nomex© or flame retardant impregnated cotton) is highly recommended when working with large quantities of flammable chemicals.

Lab coats, as far as possible, should not leave the laboratory areas. It is recommended that users who regularly attend laboratories at a distance from each other, for example on different floors, have a lab coat in each different laboratory area.

Washing the lab coat:

i) The lab coat must be carried in a well-closed disposable plastic bag;

ii) It should be washed separately from other clothes, without the use of fabric softener (increases permeability of contaminants and decreases flame retardant capacity);

(iii)a wash cycle must be run with the machine empty before returning to use with normal laundry;

(iv) if a lab coat contains massive contamination (e.g. due to spraying or spillage), it shall simply be discarded as hazardous waste;

(v)If used in biohazard work, in particular with BSL-2 microorganisms, it should be decontaminated by autoclaving before washing. When disposable lab coats are used, they should be disposed of as biological waste.

The RSL shall identify any additional situations where alternative or more protective clothing should be worn.

Eye protection

The selection of the most suitable eye protection should take into account the following elements:

- It must protect against specific hazards in the workplace;
- It must fit properly and be reasonably comfortable to wear;
- Must provide unrestricted vision and movement;
- It should be durable and clean;
- Must allow for unrestricted operation of any other PPE required.

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Safety goggles

Safety glasses have safety frames constructed of metal or plastic and impact-resistant lenses. Side protection is also a requirement.

This PPE is appropriate and should be worn by all people present while performing the following operations:

- Work without an effective barrier between the eyes and hazardous solid chemicals and small amounts of hazardous liquid chemicals;
- Work without an effective barrier between the eyes and potentially infectious materials, including microorganisms and BRL-2 viruses, human and non-human primate material, outside a biosafety chamber (in these operations a visor must also be used);
- Work without effective barrier between eyes and unsealed, liquid or powdery radioactive materials;
- Manipulation without effective barrier between eyes and particles, dust or glass fragments;
- Working without effective barrier between eyes and glass material under pressure or in vacuum;
- Cutting/connecting glass tubes;
- Replacement of compressed gas cylinders and fixing of cylinder´s regulator;
- Use of compressed air gun.

Chemical goggles

This PPE provides adequate eye protection, which completely covers the eyes and facial area around the eyes. Provides protection against impacts, dust, and liquid sprays.

This PPE is appropriate and should be worn by all those present when performing the following operations:

- Working without an effective barrier between the eyes and considerable quantities of liquid hazardous chemicals, in operations with significant projection risk, such as direct transfer of corrosive liquids;
- Work without an effective barrier between the eyes and cryogenic liquids;
- Work without an effective barrier between the eyes and highly reactive, explosive or pyrophoric materials.

Laser goggles

This PPE is required for the use of class 3 and 4 lasers, in cases where there is a possibility of irradiation of the eye. The selection of this type of PPE should take into account that each of them is designed for a certain wavelength and energy.

Contact lenses

Recent studies indicate that contact lenses do not increase the chemical risk of the eye, and can even minimize damage in many situations of eye accident.

There is, however, a short list of compounds that are unsafe to handle with contact lenses: *methylene chloride, 1,2-dibromo-3-chloropropane, acrylonitrile, ethylene oxide, dianiline methylene.*

Medical prescription glasses

This type of goggle does not offer the eye protection required by European standards. So, just like any other user, anyone who wears prescription corrective glasses will have to put safety glasses over their corrective glasses.

Gloves

The use of gloves protects against cutaneous absorption of chemicals, chemical burns, thermal burns, lacerations, biohazard and contact with cryogenic liquids.

Gloves should always be inspected for punctures and signs of degradation before starting to work with hazardous materials.

Care should be taken not to contaminate objects in the laboratory that are usually touched without gloves, such as telephones, pens, computers, or faucets. Potentially contaminated gloves should be removed before touching such objects. For the same reason, gloves should be removed when leaving the laboratory. They should not be used in hallways, elevators, or when touching door handles and buttons.

They should be used when working in a biological safety chamber, when coming into contact with biological agents (especially those that generate aerosols, and when cleaning up spills). Gloves contaminated with biological material should be placed in an autoclave bag for decontamination.

Removing the gloves

The gloves must be removed avoiding skin contact with the outside of the glove and possible contamination:

- In the case of reusable gloves, wash before removing according to the manufacturer's instructions;
- Hold the outside of a glove with the other gloved hand and carefully pull the glove from the hand, turning it from the inside out;
- With your hand without a glove, slide your finger through the opening of the other glove, avoiding touching the outside. Carefully pull the glove, turning it inside out again. Thus, all contamination is contained;
- In the case of disposable gloves, arrange for their proper disposal as hazardous waste.

Selection of suitable gloves

Selecting suitable gloves can be challenging. The gloves are available in a variety of materials, thicknesses, and with different cuff lengths to protect against different hazards.

The material of the glove and its thickness determine how resistant the glove is to a particular chemical.

Manufacturers test gloves for protection against common chemicals and often provide glove selection charts, summarizing the results of the tested parameters as follows:

- Breakthrough time (time it takes for a chemical to migrate through the glove material until it can be detected inside);
- Type of degradation (the physical change that happens to the glove when it is exposed to a chemical – e.g. cracking, swelling, hardening or shrinking);
- Permeation rate (the rate at which a chemical is absorbed by the glove and is released by the glove into its interior).

If a glove material has high resistance to one chemical, it does not mean that it is resistant to all chemicals. The manufacturer's rating and/or safety data sheets should always be checked when selecting a glove.

The various types of gloves and their applications are summarized in the following table (adapted from Division of Research Safety, Univ. of Illinois, https://drs.illinois.edu/Page/SafetyLibrary/PersonalProtectiveEquipment):

Material	Image	Features	Application
Butyl		Broad-spectrum chemical protection (consult manufacturer for specific compounds). Thick and reusable.	Large volumes of hazardous products and highly toxic liquids.
Nitrile		Good chemical resistance (consult manufacturer for specific compounds), and resistant to cutting and drilling. Reusable.	Large volumes of hazardous products and highly toxic liquids.
Viton		Good resistance to permeation of chemicals in general (consult manufacturer for specific compounds). Reusable.	Large volumes of hazardous products and highly toxic liquids.
Natural rubber		Broad-spectrum chemical protection (consult manufacturer for specific compounds). Reusable.	Can be used with a wide range of products.
Disposable Nitrile		Very limited chemical resistance.	Low-hazard products or biological material. Replace regularly.

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PHYSICAL RISK
ELECTRICAL RISKS
ELECTROMAGNETIC RADIATION
NOISE
CRYOGENIC FLUIDS
COMMON EQUIPMENT
CHEMICAL RISK
GENERAL PREVENTIVE MEASURES
PURCHASE OF HIGHLY HAZARDOUS CHEMICALS
STORAGE
SAFE TRANSPORT
FIRE PREVENTION
POTENTIALLY EXPLOSIVE COMPOUNDS
PARTICULARLY HAZARDOUS SUBSTANCES (HEALTH)
CORROSIVE SUBSTANCES
COMPRESSED GASES
LABORATORY VENTILATION
PERSONAL PROTECTIVE EQUIPMENT
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Disposible Latex		Splash protection from aqueous solutions.	For biological hazards. Not suitable for chemical use.
High Temperature Fabric		Heat resistance. No chemical resistance.	Handling hot objects.
Inflated		Water resistance. No chemical resistance.	Handling of cryogens.
Metallic Textile		Cut resistance. No chemical resistance.	Handling sharp objects such as blades, broken glass, or needles.
Leather		Abrasion and heat protection.	Handling hot objects. Mechanical work.

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2.8 HAZARDOUS WASTE MANAGEMENT

FCUP is legally responsible for the management of its waste while it is inside the Campus, as well as for transport and processing, if damage occurs due to failures in packaging/content. It is therefore necessary to lay down rules for their management, up to the time of collection by duly licensed operators. The MSL only describes the general principles of hazardous waste management. For details regarding the collection processes, labelling, documentation and for clarification of doubts, please contact:

Department of Biology (DBIO): Teresa Mendes (teresa.mendes@fc.up.pt; ext. tel. 70721)

Department of Physics and Astronomy (DFA): Armanda Silva (masilva@fc.up.pt; ext. tel. 40433)

Department of Geosciences, Environment and Spatial Planning (DGAOT): Paulo Ferreira (paferreira@fc.up.pt; ext. tel. 40489)

Department of Chemistry and Biochemistry (DQB): Cláudia Alves (claudia.alves@fc.up.pt; ext. tel. 30642) / Mafalda Flores (mafalda.flores@fc.up.pt; ext. tel. 30629)

Waste is separated on the basis of the European List of Waste, LER, published by Decision 2014/955/EU, which concerns a harmonised list of waste that takes into account its origin and composition.

Chemical waste

Only those chemicals that are undoubtedly completely harmless to people, sewers, pipes, wastewater treatment processes, open water and their organisms, both in the short and long term, can be disposed of directly into the sewer or urban waste system. Safety data sheets or other reliable sources of information should be consulted, and in case of doubt, the product should be treated as hazardous waste.

Hazardous chemical waste is packed in containers of appropriate size and constitution, distributed by the departmental services.

Containers must be labelled with information on the hazards contained, the name of the waste, the place of production and the date of the start of collection. Hazards must be indicated by means of the appropriate pictograms and in full.

Label example (in Portuguese):

Perigo

Local: FC2 0.11

Responsável: Nome Apelido

Data Inicial: DD/MM/AAAA

LER 16 05 06* UN2810

Resíduos de laboratório

Advertências de Perigo:
H370 Afecta os órgãos. **H314** Provoca queimaduras na pele e lesões oculares graves. **H410** Muito tóxico para os organismos aquáticos com efeitos duradouros.
Recomendações de Prudência:
P260 Não respirar as poeiras/fumos/gases/névoas/vapores/aerossóis. **P280** Usar luvas de protecção / protecção ocular. **P303+P361+P353** SE ENTRAR EM CONTACTO COM A PELE (ou o cabelo): retirar imediatamente toda a roupa contaminada. Enxaguar a pele com água/tomar um duche. **P305+P351+P338** SE ENTRAR EM CONTACTO COM OS OLHOS: enxaguar cuidadosamente com água durante vários minutos. Se usar lentes de contacto, retire-as, se tal lhe for possível. Continuar a enxaguar. **P501** Eliminar o conteúdo/recipiente de acordo com a legislação local/regional/nacional/internacional.
O produto contém: Resíduos de Laboratório

Containers may only be filled up to the mark indicated on the front.

After each use, they must be properly closed, avoiding the release of vapors or aerosols.

In order to avoid the outbreak of toxic/foul-smelling vapors elsewhere in the building, after the rejection of a toxic and/or disturbing smelling product, the residual of the spilled product must be washed and collected in the waste container, using small portions of a less harmful solvent, such as ethanol, for example.

Containers that are not properly closed, identified, or with a volume exceeding the mark will not be accepted in waste warehouses.

The disposal of obsolete or no longer in use solids should be carried out preferably by dissolution in an appropriate solvent (lower costs). If this is not possible, they must be placed in suitable boxes (consult the departmental services) with the identification of the products to be discarded, when known, as well as the pictograms associated with the hazardousness of the products.

Some of the LER items most commonly used to classify laboratory chemical waste are:

- Code LER 06 04 05 - Waste containing heavy metals;
- Code LER 14 06 02 - Other solvents and mixtures of halogenated solvents;
- Code LER 14 06 03 - Other solvents and mixtures of solvents (non-halogenated);
- Code LER 15 01 10 - Packaging containing or contaminated by residues of hazardous substances;
- Code LER 15 02 02 - Absorbents, filter materials, cleaning cloths and protective clothing, contaminated by hazardous substances (e.g. contaminated absorbents, fume hood filters, PPE);
- Code LER 16 05 06 - Laboratory chemicals containing or composed of hazardous substances, including mixtures of laboratory products;
- Code LER 16 05 07 - Inorganic laboratory chemicals containing or composed of hazardous substances.

IMPORTANT NOTES:

- Certain products cannot be mixed, although they may be classified in the same broad waste category. For example, "Inorganic laboratory chemicals containing or composed of hazardous substances" can include many products that are incompatible, such as ammonium nitrate and various perchlorates. Therefore, it is essential to consult the safety data sheet of each product before disposal (section 10);

- Some ethers are common solvents and precursors of peroxides. The same precautions described in **Acquisition, handling and storage of potentially explosive compounds** should be applied to this waste.

Biological waste

Biological waste includes all biological material (e.g. cell cultures/residues, including microorganisms) developed/manipulated in the laboratory, and all objects potentially contaminated by contact with such material.

Proper disposal of biological waste is essential to avoid risks to human and animal health, contamination of laboratories/infrastructures, and environmental contamination. Contaminated material cannot be disposed of in regular garbage or drains.

Some of the LER items most commonly used to classify laboratory biological waste are:

- Code LER 18 01 01 - Sharp objects (except 18 01 03);
- Code LER 18 01 02 - Anatomical parts and organs, including blood bags and preserved blood (except 18 01 03);
- Code LER 18 01 03 - Waste whose collection and disposal are subject to specific requirements for the prevention of infections;
- Code LER 18 01 06 - Chemicals containing or composed of hazardous substances;
- Code LER 18 01 08 - Cytotoxic and cytostatic drugs.

Hazardous biowaste is further divided into the following risk categories:

GROUP III – solid or liquid waste that has not come into contact with hazardous drugs or chemical reagents. This waste will then be treated by autoclaving. If the waste is not collected by an external company, the decontamination process is described in more detail in this Manual (see Autoclaving Decontamination, below).

GROUP IV - solid or liquid waste that has come into contact with dangerous drugs or chemical reagents (including bleach). This waste will then be treated by incineration, so its treatment is always guaranteed by an external company.

IMPORTANT NOTES:

- Waste containing formol must be placed in appropriate containers (it must not be mixed with other waste), and must be collected by a specialized company as group IV waste.

- Carcasses of unpreserved animals must be kept at -20 °C in a freezer exclusive for this purpose until the moment of collection by a specialized company, being collected as group IV waste or as M1 animal by-product (provided that they are not manipulated) (commercially purchased carcasses are excluded).

- Microbiological and histological preparations should not be mixed with other contaminated glass material, and should be placed in appropriate containers for collection by a specialized company as group IV waste.

- Sharp and piercing objects, such as syringes and scalpel blades, regardless of the nature of the contamination (biological, chemical, mixed or non-existent), must be disposed of in sharp-piercing containers (group IV), resistant to their cutting/piercing action. To avoid injury, the needles should be separated from the syringes using the adapter on the lid of the container.

- Contaminated and absorbent PPE should be treated as group III or IV solid biological waste, depending on the type of contaminant to which they have been exposed.

The transport of waste from the production site to the decontamination or storage site should be done on trolleys and never by hand, so as to avoid accidental spillage.

Autoclaving decontamination

The decontamination of biological waste by autoclaving is done in autoclaves that are intended for this purpose only. The effectiveness of autoclaving should always be validated by standardized indicator systems.

They may only be operated by trained personnel.

The basic rules of use can be found as **annex**.

Recycling of laboratory containers

The collection of recyclable glass (non-pyrex or non-borosilicate), plastic or metal containers, even if they contained hazardous substances, will be carried out on the assumption that the material is properly decontaminated, and the label and caps are removed.

The decontamination process involves the complete emptying of the containers (drained or scraped to the maximum extent) and the removal of the residual waste film. Residual removal can be carried out in different ways, depending on the substances:

(i) overnight fume hood evaporation for volatile and semi-volatile liquids that do not present high respiratory and dermal toxicity (e.g. ethanol or acetonitrile);

(ii) rinsing the entire inside of the container (at least 3 times) with small quantities of a solvent of the type referred to in (i), followed by procedure (i) (evaporation), for highly toxic liquids such as dichloromethane or methanol and for non-water-soluble solids;

iii) rinse with water (at least 3 times) for water-soluble solids.

All rinsing liquids should be collected as hazardous chemical waste.

In case of doubt, please consult the departmental services, and if any doubt about the effectiveness of decontamination persists, the container should be disposed of as hazardous waste.

In addition to containers, non-contaminated packaging protection plastic is also accepted.

The rest of the laboratory material should be disposed of as hazardous waste, even if it is not contaminated (see image below).

The presence of undue laboratory waste in municipal waste, if detected, may result in the refusal of collection and the imposition of fines on the waste producer.

Radioactive waste

The disposal of radioactive waste requires more complex procedures, which involve the Portuguese Environment Agency, the Nuclear Technological Campus, the collection company and UP. Contact CCSL (ccsl@fc.up.pt) in case of need to dispose of radioactive waste.

3.1 EMERGENCY SITUATION PROCEDURES

Internal Emergency Contacts

In case of emergency, and only in that case, call one of the numbers corresponding to the building you are in.

INTERNAL EXTENSION FCUP	#112
SECURITY POST FC1	220 402 112 | 963 605 071
SECURITY POST FC2/FC3	220 402 212 | 967 180 422
SECURITY POST FC4/FC5	220 402 412 | 967 180 447
SECURITY POST FC6	220 402 612 | 967 180 448
	Save these numbers!

External emergency contacts

EUROPEAN EMERGENCY NUMBER	112
POISON INFORMATION CENTER (CIAV)	800 250 250
PORTO FIREFIGHTER BRIGADE	225 073 700
Santo António Hospital	222 077 500
Police - Foz	226 153 010
Porto Municipal Civil Protection Service	222 071 310

Refer to the "Emergency" page in Sigarra FCUP, accessible from the home page in the menu on the right, for more details.

Internal Emergency Plan – Fire

The Internal Emergency Plan (EIP) indicates the self-protection measures to be adopted to deal with a fire situation in the premises, namely the organization, the human and material resources to be involved, and the procedures to be followed in such a situation.

The common user of laboratories should have a minimum knowledge of the EIP, in particular:

- The existence of a Safety Service (SS) consisting of a command unit and action teams (evacuation, 1st intervention, first aid, among others). The constitution of the SS of the building where the laboratory is located must be known to all;
- Knowledge of the fire protection equipment present in the laboratories or in the vicinity (see images below);
- The procedure for action in the event of a fire breaking out in the workplace;
- The evacuation procedure in case of emergency;
- Relevant safety signage (see below).

Flame retardant blanket

Fire extinguisher

Emergency shower

Reel (for exclusive use by SS personnel)

It is of utmost importance:

- To know the location of safety equipment and ensure its easy access, making sure there are no obstructions to it;
- Inform the RSL in case any of the equipment is not present or is not in good condition.

In case of fire:

- Stay calm and notify the Security Post (preferably) or activate the nearest manual alarm panel;
- Alert other occupants for help and for the possible need to evacuate the affected area;
- If clothing is on fire, do not run: cover the burning area with a flame retardant blanket or lie down on the floor, rolling over on yourself. Alternatively, use the nearest emergency shower;

- In the event of smoke, protect your mouth and nose with a cloth and stay close to the ground, where there is less smoke and more oxygen;

- If the person feels able to intervene, fight the fire using a flame retardant blanket or a fire extinguisher, without putting themselves in danger. Consult the instructions for use of the **flame retardant blanket** and **fire extinguishers**;

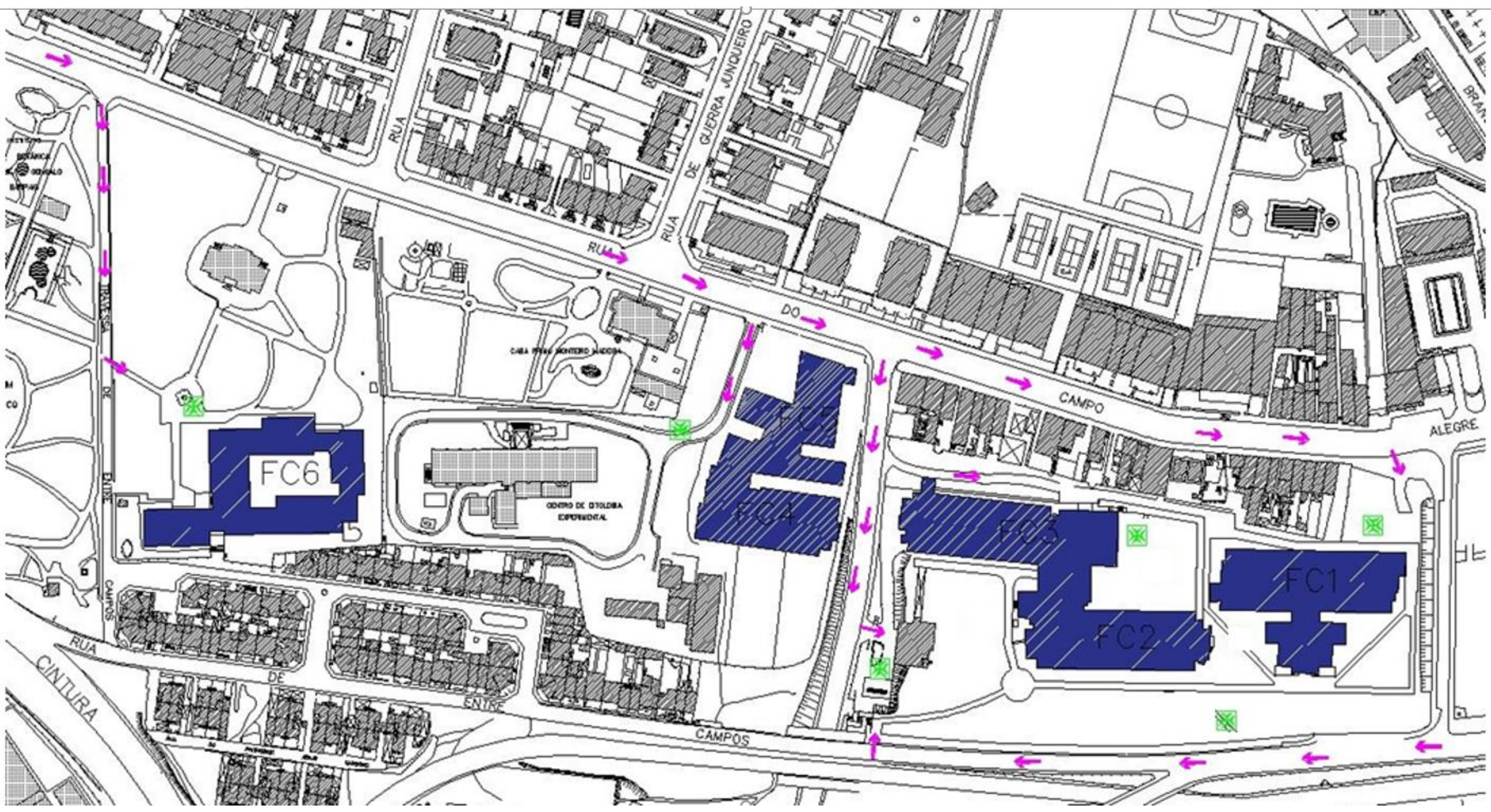
- If the fire originates from electrical equipment, carry out a local power cut if this is safe;

- If the fire cannot be extinguished, the place should be abandoned, if possible closing the doors and windows.

The general alarm consists of the activation of the siren of the Automatic Fire Detection System (SADI) continuously. The activation of this alarm implies the evacuation of the building using the existing escape routes for this purpose, following the indications of the evacuation signs. Evacuation may also take place without an audible alarm, if ordered by the evacuation team or other members of the SS.

Watch **here** a video illustrating how to act in case of evacuation at FCUP.

The following map shows the location of the various meeting points on the FCUP Campus, for the gathering of occupants at the end of the evacuation, as well as the access routes for emergency vehicles:



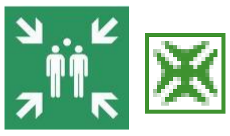
☒ Meeting point

The purpose of safety signs is to inform and draw attention, in an efficient and unambiguous manner, to objects and situations that may cause danger, as well as to remind the appropriate instructions and procedures in specific situations.

Emergency Signs:

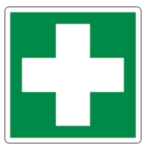
Direction to follow

Passage opening



Meeting point

Shower/ eye-wash



First aid box

<u>Fire Fighting Equipment Signs:</u>


Fire extinguisher


Fire retardant blanket


Panel


Reel


Fire hose

Acting in the event of an accident.

In the event of a major accident (other than fire) that endangers the integrity of the building and/or its occupants, such as a serious chemical or biological spill, explosion or release of a dangerous gas, the entire building must be evacuated. In this situation, it is important:

- Stay calm and notify the Security Post (preferably), or activate the nearest manual alarm panel;
- Alert other occupants for help and for the possible need to evacuate the affected area;
- Leave the premises, if possible by closing the doors and windows.

In the event of an <u>accident that results in a medical emergency</u>:

i) Before rescuing an injured person, one must verify that he is in a position to do so without jeopardizing one's own safety;

ii) Do not move the victim, except if strictly necessary, when he or she may be in even greater danger by inhalation or other type of exposure;

iii) Inform the Security Post, to activate the 1st aid team and INEM, or as a last resort, call directly the European emergency number, 112, immediately informing the Security Post;

iv) If you call 112, the person making the contact must inform that a medical emergency has occurred, give their telephone number, the exact place where they are (Faculty of Sciences of the University of Porto, address, department, floor/room), indicate how the victim is, if there has been any type of action, and calmly answer the questions put to him. You should only hang up the call when you receive an indication from the operator of line 112.

This procedure should be adopted in serious accidents, such as those involving deep blows, severe burns, inhalation of toxic substances, loss of consciousness, cardiorespiratory arrest, convulsions, fractures, state of shock, etc.

In the event of an <u>accident with minor consequences</u>:

- Inform the Security Post, so that the 1st aid team can be activated, or alternatively contact a member of that team directly.

The SS 1st aid teams are properly trained and better equipped, compared to the products and utensils present in the 1st aid boxes of the laboratories.

However, certain types of accident or minor health problems require immediate intervention by the injured person himself or any other common user of the laboratories. Some basic procedures are presented in the following table.

Type of Accident	Procedure
Wounds	• If the wound is not very deep, let it bleed for a few seconds. • Remove small foreign bodies. Deep-set objects should not be removed. • Wash the wound thoroughly with saline solution; • Protect with a sterile swab soaked in saline solution and cover with a ligature or bandage, depending on the size.
Minor non-chemical burns (1st degree)	• Remove the victim from the cause or the cause from the victim. • Add lukewarm running water (not cold) for 10 - 20 min. • Cover the burned areas with compresses moistened with saline solution or water. • Do not remove anything that is stuck to the burnt skin. • Contact the Security Post if medical assistance is required
Major burns	• Request help. • Rinse the affected area thoroughly with water. • Refer for urgent medical care. •
Chemical burns (ocular route)	• Identify the product that caused the injury. • Rinse the eyes thoroughly with the eyelids open with saline, borate solution or in the eyewash station. Cover the eye without pressure and refer for urgent medical assistance.
Chemical burns (cutaneous route)	• Identify the product that caused the injury. • Rinse thoroughly with saline solution. • Cover the burned areas with compresses moistened with saline solution or water. • Refer for urgent medical care.

Type of Accident	Procedure
	Important Note: there are exceptions to these rules. With some acids or bases (see SDS) it is advisable to wash with basic or acidic solutions, as the case may be. Acid burn: (wash with 5% Na_2CO_3 or equivalent solution). Alkali burn: (wash with 5 % acetic acid or equivalent solution)
Projection of chemicals onto the body or clothing	• Identify the chemical. • Remove the victim from the cause or the cause from the victim • Remove the affected clothes. • Wash the body thoroughly with water for at least 15min (emergency shower). • Refer for medical assistance.
Projection of chemicals into the eyes	• Identify the chemical. • Immediately wash the face and eyes in the eyewash station for at least 15 min. • Refer for medical assistance
Broken glass	• Never touch the glass with bare hands. • Sweep into a suitable container with the help of a shovel or paper towels. • If a chemical is in the glass, dispose of it in a proper and identified container (contaminated waste)
Small chemical spill (up to 5 L)	• Aerate the area in the case of volatile chemicals, especially if flammable or toxic. • In the case of hazardous liquids, contain and collect the spill using one of the existing kits (**instructions for use here**). • Wash the area, ensuring that all the chemical has been removed
Spills of biological material	• According to the site of the spill (biological safety chamber, other laboratory surfaces, inside centrifuges or incubators) appropriate procedures must be adopted (**see annex**). Alert the teacher/researcher in charge of the laboratory
Exposure to biological agents with a risk of infection (e.g. cracked or cut skin, contaminated needle prick)	• Wash the affected area thoroughly with soap and water and then disinfect. In case of contact with the eyes, they should be washed thoroughly in the eyewash station or with saline solution.
Small fire outbreak	• Move the container away from ignition sources. • Use a flame retardant blanket. • If necessary, contact the Security Post
Electric shock	• Cut off or turn off the power source, but do not touch the victim. • Move the victim away from the electrical source that was causing the shock, using non-conductive and dry materials such as wood (broomstick), plastic, thick cloths or rubber. • Contact the Security Post.
Feeling faint (no loss of consciousness)	• Sit the victim with the head between the legs or • Lay the victim down with the head lower than the legs. • If necessary, unfasten the clothes

3.2 Accidents and Near Misses (internal procedure)

An accident is an unintentional occurrence that has caused injury or other damage. An injury is an effect on the body as a result of the accident, for example, a cut finger, a sprained ankle, or a chemical burn. Accidents occur when hazards are not identified during the definition of preventive measures, when risks are not obvious, or as a result of combinations of circumstances that are difficult to predict.

Sometimes, accidents almost happen, but luckily, for some fortunate reason, no damage is done. This is referred to as a near miss. In other cases, the damage may not be immediately noticeable.

The recording and analysis of any of these types of occurrences is of paramount importance for the improvement of prevention measures in an organization. The recording and analysis of accidents and near misses should be carried out as soon as possible, but obviously giving priority to any medical or other emergency action.

Objectives and type of approach

A thorough analysis of accidents/near misses can identify previously overlooked physical, environmental, administrative, or process risks, the need for new or more extensive safety training, or unsafe work practices. **The goal of any accident analysis should be to determine the causal factors surrounding the occurrence and to recommend corrective actions to prevent similar occurrences in the future**. Those responsible for the analysis should avoid any emphasis on trying to identify someone as being at fault for what happened, as this threatens the credibility and effectiveness of the accident analysis process. However, this does not mean that relevant inspections, unsafe acts, or omissions by employees, supervisors, or others should be ignored.

Guidelines for recording and analysing incidents

Filling out the Accident or Near Miss Report Form

When a laboratory user is involved in an accident or near miss, the RSL or their supervisor must complete an Occurrence Report Form.

Analysis of the occurrence

Each accident/near-miss offers the possibility of preventing a similar one from occurring in the future. The RSL or supervisor can play an important role in this process by accurately completing the occurrence report form.

The analysis should begin as soon as possible after the occurrence is reported. The RSL or the supervisor of the person or persons involved must initiate the analysis in order to accurately complete the form. By starting as soon as possible, the facts and conditions associated with the occurrence will be clearer to those present at the time of the accident.

The occurrence report form must be submitted to the safety committee of the respective department, which, if necessary after further review, will provide a proposal for the correction of any unsafe practices or conditions identified. This proposal should be submitted to the Coordinating Committee for Safety in Laboratories to allow the entire FCUP to benefit from the knowledge resulting from the occurrence analysis.

Departmental safety committees are encouraged to request the collaboration of external elements in the analysis of occurrences, namely from other departmental committees, the Coordinating Committee for Laboratory Safety, the Safety, Hygiene and Sustainability Unit (USHS) or others, whenever they consider it useful or necessary.

3.3 External Accident Reporting / Insurance

The reporting of accidents and occupational diseases to the competent external entities must be carried out through the Human Resources Service (SRH) of FCUP, within the deadlines provided for in the legislation and policies referred to below.

The "Compensation Scheme for Accidents at Work and Occupational Diseases" - Law No 98/2009 - establishes the conditions under which workers are entitled to compensation for damage resulting from accidents on duty and occupational diseases.

Some concepts and key points

Occupational accident - one that occurs at the place and time of work and directly or indirectly produces bodily injury, functional disturbance or illness resulting in a reduction in the ability to work or to earn or death.

Occupational disease - bodily injury, functional disturbance or disease that is a necessary and direct consequence of the activity performed by the worker, and does not represent normal wear and tear of the body.

Incident - any event that affects a particular worker, in the course of work or related to it, which does not result in bodily injuries diagnosed immediately, or in which they only require first aid.

Dangerous event - any event easily recognised to constitute a risk of accident or illness for workers in the course of work or for the general public.

The employer is responsible for compensation and other charges arising from an accident at work or an occupational disease, whether of a singular or collective nature, under private or public law, provided that it is not covered by special legislation.

The employer does not have to repair the damages resulting from the accident that is intentionally caused by the victim or results from his act or omission, which involves a violation, without justifiable cause, of the safety conditions established by the employer or provided for by law.

Employees with an employment contract within the Public Administration

The special legislation "Legal Regime of Accidents in Service and Occupational Diseases within the Public Administration" - Decree-Law No. 503/99 - applies.

Points to highlight:

- "The service or body of the Public Administration at the service of which the accident occurred or the occupational disease was contracted is responsible for the costs of repairing the damages arising therefrom, under the terms provided for in this law. In cases where there is permanent incapacity or death, it is incumbent upon the Caixa Geral de Aposentações to assess and repair it, under the terms provided for in this diploma";

- "The employee linked to the Administration who, fraudulently, tries to benefit or benefits from any protection or reparation provided for in this diploma incurs in a disciplinary infraction punishable by the penalties of suspension or inactivity, according to the seriousness of the infraction, under the terms of the Disciplinary Statute";

- "In the event of an accident, the employee, by himself or through an intermediary, must report it, in writing or verbally, within two working days to the respective hierarchical superior, unless the latter has witnessed it. If an incident occurs, the employee must report it in writing to his or her immediate superior within two working days. The dangerous event is reported to the employer.

- Occupational safety and health services shall:

  i) propose and organise means for the provision of first aid;

  (ii) analyse the causes of on-the-job accidents, occupational diseases, incidents and dangerous events and propose the corresponding preventive measures;

  (iii) compile statistics relating to the events referred to in the preceding paragraph;

  iv) prepare reports on accidents on duty that have resulted in an absence of more than three working days.

Employees with an employment contract

Liability for damage resulting from accidents at work and occupational diseases is transferred to an insurer through the conclusion of an insurance contract against accidents at work (employees). Consult the conditions of the contract in force at UP in the SRH.

Point to highlight:

- In the event of an accident at work resulting from failure to comply with the rules on safety and health at work, due to mere negligence or the effect of violation of a legal or regulatory rule, the insurer waives the duty to compensate for the damages.

Other individual contributors

Liability for damage resulting from accidents at work and occupational diseases is transferred to an insurer through the conclusion of a personal accident insurance contract. Consult the conditions of the contract in force at UP in the SRH.

Point to highlight:

- Intentional or grossly negligent actions or omissions by the Insured Person, Policyholder or Beneficiaries are always excluded from the scope of all insurance coverage

Students

Students benefit from a personal accident insurance that guarantees the coverage of expenses incurred in the exercise of school and sports activities at UP. See all the information here.

1. General organizational aspects
2. Access/risk management
3. Accidents/ Emergency
Abbreviations
Credits
Attachments

List of Abbreviations and Acronyms

ACT	Authority for Working Conditions
APA	Portuguese Environment Agency
AVAC	Heating, Ventilation and Air Conditioning
BSL	BioSafety Level
BRL	BioRisk Level
CCSL	Laboratory Safety Coordinating Committee
CDC	Centers for Disease Control and Prevention
CIAV	Poison Information Center
CLP	Classification, Labelling and Packaging
CS	Safety Committee
CSB	Biological Safety Chamber
DGS	Directorate-General for Health
PPE	Personal Protective Equipment
FCUP	Faculdade de Ciências da Universidade do Porto (Faculty of Sciences of the University of Porto)
FFI	Individual Training Sheet
SDS	Safety Datasheet
LER	European List of Waste
MAP	Self-Protection Measures
MGM	Genetically Modified Microorganism
MSL	Laboratory Safety Manual
OGM	Genetically Modified Organism
PEI	Internal Emergency Plan
REACH	Registration, Evaluation, Authorization of Chemicals
RSL	Laboratory Safety Officer
SABA	Alcohol-Based Antiseptic Solution
SADI	Automatic Fire Detection System
SCIE	Fire Safety in Buildings
SILiAMB	Integrated Environmental Licensing System
SIMS	Infrastructure, Maintenance and Sustainability Services
GHS	Globally Harmonized System
SRH	Human Resources Service
SS	Safety Service
TACMR	Acute Toxicity, Carcinogenic, Mutagenic and Reprotoxic
UC	Course Unit
USHS	Safety, Hygiene and Sustainability Unit

CREDITS

This manual was prepared and proposed by the Coordinating Committee for Laboratory Safety (CCSL), consisting of Carla Rosa (member of the Executive Committee of FCUP, Professor at DFA), Cláudia Alves (Senior Technician at DQB), Francisco Carpinteiro (Senior Technician at DFA), Helena Brites (Professor at DGAOT), Joana Silva (Senior Technician at DB), Manuel Azenha (Pro-director for Safety and Health at Work, Professor at DQB), Manuel Marques (Professor at DFA), Paula Melo Gomes (Professor at DB) and Paulo Ferreira (Senior Technician at DGAOT).

In addition to the legislation referred to in the manual, it resulted to a large extent from a synthesis and adaptation to the reality of FCUP, based on several documents available online, of which manuals from other university institutions stand out, namely:

- Princeton University (**https://ehs.princeton.edu/laboratory-research/laboratory-safety/laboratory-safety-manual**);
- University of Washington (**https://www.ehs.washington.edu/resource/laboratory-safety-manual-510**);
- University of Illinois (**https://drs.illinois.edu/site-documents/LaboratorySafetyGuide.pdf**);
- Instituto Superior Técnico (**https://nshs.tecnico.ulisboa.pt/files/sites/10/manual-de-seguranca-para-laboratorios.pdf**);
- Universidade de Aveiro (**https://www.ua.pt/pt/dqua/page/5313**).

The CCSL would like to thank all those who contributed to the preparation of this manual, through discussions, suggestions and in other ways, in particular:

- To the Director of FCUP, Professor Cristina Freire, and her Executive Committee, for the revisions and suggestions provided, in particular to Professor Conceição Santos for her important contribution to the chapter on biological risk, and to Professor Lucinda Lima for her decisive contribution in the structuring and organization of the document;
- To Engineers Nuno Fontoura and Paulo Santos (SIMS), for their contributions related to infrastructure, ventilation and emergency procedures;
- To Senior Technician Mafalda Flores, for her contribution to the management of hazardous waste.

ATTACHMENTS

- Declaration of commitment to comply with the safety procedures defined in the FCUP Laboratory Safety Manual;
- Individual Training Sheet - FCUP;
- Autoclave decontamination procedure;
- BSL-2 Laboratories identification template, to be affixed to the door of laboratories where BRL-2 microorganisms are handled (classes and research);
- Acting in case of spills of biological material;
- Hand washing and disinfection of surfaces;
- List of common incompatible compounds;
- Instructions for Use - Flame Retardant Blanket;
- Instructions for Use - ABC Chemical Powder Fire Extinguisher;
- Instructions for Use - CO_2 fire extinguisher;
- Instructions for Use - Spill Containment and Removal Kit;
- Instructions for Use - Filtering Mask;
- Occurrence Report Form (accident or near-accident).

DECLARATION OF COMMITMENT TO COMPLY WITH THE SAFETY PROCEDURES DEFINED IN THE FCUP LABORATORY SAFETY MANUAL

I ____

number/ Type of identification ____________________/___________________

1. I declare that I have read the FCUP Safety Manual for Laboratories, as well as the specific addendums of laboratories ____, having understood the content of the rules applicable to my activity, namely the safety rules, the rules of conduct and use of spaces, equipment and materials;

2. I declare that I undertake to comply with the rules, indications and methodologies defined in the Laboratory Safety Manual and in the addendums referred to in 1., as well as to ensure that they are complied with;

3. I declare to be aware and agree that any non-compliance or infraction of the standards, guidelines or procedures established in the Laboratory Safety Manual represents a transgression of the code of ethics of the University of Porto. I recognize that such a violation constitutes a breach of the duties that, according to the legislation and regulations in force, all members of the academic community must observe in relation to the University. I am informed that this may lead to disciplinary consequences in accordance with the provisions of the applicable regulations of the U.Porto.

Porto, ______ of __________________ of 20____

FCUP User Signature: ____

Received by (Laboratory Safety Officer): _______________________________________

Note:

The applicability of this declaration to short-term users (up to 2 weeks) is at the discretion of the respective supervisor/host, who will be entirely responsible for the actions taken by this type of user.

User name: Laboratory Safety Officer (RSL):

Laboratory(ies):

Mandatory training components (if applicable)

Training	Date	User signature	Name and Heading of the trainer
Check-in			/
Information on the MSL and collection of the declaration of commitment			/
Practices adopted regarding solitary work and unaccompanied experiences.			/
Effective knowledge of the laboratory regarding the location of firefighting equipment, emergency exits, spill containment kits, etc.			/
Practices adopted in the identification and labeling of materials, reagents, samples, residues, etc.			/
Management of all hazardous waste produced.			/
Safe use of each of the necessary equipment.			/
Practices adopted in the previous preparation of a new experimental work.			/
Obtaining appropriate PPE and disposing of it.			/

Mandatory components in case of existence/use of hazardous substances, BSL ≥ 2 or BSL-1 measures with specificities justifying training, of major physical hazards as described in the MSL.

Training	Date	User signature	Name and Heading of the trainer
			/
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This sheet should be available for consultation in the laboratories or through the RSL.

AUTOCLAVE DECONTAMINATION PROCEDURE

In general, the following practices should be observed in the autoclaving process:

a. The efficiency of sterilization must be periodically confirmed by chemical or, preferably, biological methods and recorded;

b. Materials that may generate toxic vapors (e.g. bleach, solvents, volatile reagents) should not be autoclaved;

c. Appropriate PPE should be worn when loading and unloading the autoclave;

d. Do not use perforated baskets and use only bags suitable for autoclaving;

e. In order to ensure steam penetration, autoclave bags should be left partially open, and containers containing liquids should have their lids partially open;

f. When loading the autoclave, the load must be arranged in such a way as to allow steam to circulate and not to touch the lid;

g. Keep equipment surfaces clean;

h. Make sewage at least once a week or whenever necessary;

i. Carry out general cleaning at least twice a month or whenever necessary;

j. Any irregularity detected must be reported to the responsible technician.

Specific procedure for solid biological waste:

(i) place the material to be decontaminated in plastic bags suitable for autoclaving up to approximately 3/4 of its capacity;

ii) autoclave;

iii) after autoclaving, the bags containing the decontaminated biological waste can be discarded; The material to be reused can be washed.

Specific procedure for liquid biowaste:

i) place in containers resistant to autoclaving, not exceeding half of their capacity;

ii) autoclave;

iii) after decontamination, pour the liquids into jerrycans to be collected by a specialized company as group III waste;

(iv) wash or dispose of containers such as glass or laboratory plastic.

As an alternative to autoclaving liquid biowaste, bleache at a final concentration of 20 % can be added and waited at least 30 minutes before disposal (as group IV biowaste).

However, the addition of bleach cannot be used for contaminants that have forms of resistance, such as spores (autoclaving is mandatory).

Note: *In this manual, the bleach concentration refers to the percentage of dilution from the commercial product (bleach with active chlorine concentration between 25 and 50 g/L).*

BSL-2 LABORATORIES IDENTIFICATION TEMPLATE, TO BE AFFIXED TO THE DOOR OF LABORATORIES WHERE BRL-2 MICROORGANISMS ARE HANDLED (CLASSES AND RESEARCH)

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO
DEPARTAMENTO DE BIOLOGIA

LABORATÓRIO BSL-2
ACESSO LIMITADO A PESSOAL AUTORIZADO

Responsável de Segurança do Laboratório: ____

Agentes manipulados: ____

____

Requisitos antes da entrada/saída do laboratório:

Uso obrigatório de bata no interior do laboratório (e outro EPI apropriado).

Remoção de EPI e lavagem de mãos antes de abandonar o laboratório.

Em caso de acidente contactar:

Função	**Nome**	**Contacto**
Responsável de Segurança do Laboratório		

ACTIONS IN THE EVENT OF SPILLS OF BIOLOGICAL MATERIAL

Spills inside biological safety chamber:

- Keep the CSB up and running;
- Remove contaminated PPE and place in an autoclave bag for decontamination and disposal or washing;
- Wash your hands and put on new PPE (gown and gloves) and gather the necessary material to clean up the spill (or the biological spill kit);
- Cover the spill with absorbent paper, soak it in disinfectant (20% bleach), from the periphery to the center, taking care to avoid the formation of aerosols. Leave on for 30 minutes;
- Broken glass or other debris should be removed with tweezers to the cutter-perforating container or to an autoclave bag for autoclave decontamination;
- Clean up the spill and put all the absorbent material inside a plastic bag inside the chamber, and dispose of it in the common trash;
- Remove all bleach residues with water, in order to avoid corrosion of the stainless steel;
- The remaining material that was inside the CSB at the time of the spill must be disinfected with 70 % alcohol or placed in an autoclave bag to be decontaminated.
- The interior of the CSB must be disinfected with 70% alcohol before resuming work.

Spills on laboratory surfaces, outside the CSB:

- In the event of a spill with a risk of aerosol formation, all laboratory occupants must leave the premises for at least 30 minutes;
- Request help if needed;
- PPE and contaminated clothing should be removed and placed in an autoclave bag for autoclave decontamination, and hands should be washed thoroughly. In the event that the footwear has been hit, avoid contaminating clean areas before removing them;
- Put on new PPE (lab coat and gloves) and gather the necessary material to clean up the spill;
- Cover the spill with absorbent paper, soak it in disinfectant (20% bleach), from the periphery to the center, taking care to avoid the formation of aerosols. If there is a chemical in the sample that can react with sodium hypochlorite, use 70% alcohol;
- Leave on for 30 minutes;
- Broken glass or other debris should be removed with tweezers to the cutter-perforating container or to an autoclave bag for autoclave decontamination;
- Clean up the spill and put all the absorbent material inside a plastic bag and dispose of it in the common trash;
- Material in the vicinity of the spill should be disinfected with 70 % alcohol or placed in an autoclave bag to be decontaminated by autoclaving.

Spills inside a centrifuge or incubator:

- In case of equipment malfunction or poor packaging and breakage of glassware, there is a risk of contamination by aerosol formation;
- The equipment must be switched off and kept closed for 30 minutes. Warn other personnel in the laboratory so that no one opens the equipment during that time;
- Put on appropriate PPE (lab coat, gloves), open the equipment and remove debris with tweezers. Broken glass and other waste should be placed in an autoclave bag and decontaminated by autoclaving;
- Disinfect the inside of the equipment and accessories with 20% bleach;
- Leave on for 30 minutes and remove all disinfectant residues with water to prevent corrosion of the material.

***Note:** In this manual, the bleach concentration refers to the percentage of dilution from the commercial product (bleach with active chlorine concentration between 25 and 50 g/L).*

Hand washing:

1. Wet your hands with water and apply enough soap to cover the entire surface of your hands and wrists;
2. Rub your hands vigorously for at least 15 seconds;
3. Rinse your hands thoroughly;
4. Do not touch the tap after hand hygiene (e.g. use a paper towel to turn off the tap)
5. Dry your hands thoroughly with a single-use wipe.

Friction disinfection with SABA (hand sanitiser with a minimum of 70% alcohol):

1. Follow the manufacturer's instructions for SABA application times;
2. Apply enough SABA to cover both hands on all surfaces and wrists;
3. Rub your hands vigorously for 20 to 30 seconds, until the SABA has completely evaporated, ensuring that your hands dry.

Surface disinfection

1. Wipe the worktop with a soaked material or spray it (e.g. 70% ethanol) and, after an action time of about 2 minutes, wipe with absorbent paper;
2. In spillage situations, where disinfection must be carried out with particular care, preference should be given to the 10 % bleach solution and act as described in the MSL in Accidents and spills of biological material;

To disinfect surfaces, 70% ethanol or 10% bleach can be used. However, it should be taken into account that bleach corrodes stainless steel, so when this disinfectant solution is used, the surface should be rinsed thoroughly. For routine disinfection of surfaces, preference should be given to 70 % ethanol, which should be available in all laboratories.

Note: *In this manual, the bleach concentration refers to the percentage of dilution from the commercial product (bleach with active chlorine concentration between 25 and 50 g/L).*

LIST OF COMMON INCOMPATIBLE COMPOUNDS

(Non-exhaustive list)

Chemical	Incompatibilities
Acetic Acid	Chromic acid, hydroxides, nitric acid, perchloric acid, peroxides, permanganates
Acetone	Concentrated nitric acid, sulfuric acid, and mixtures containing them
Acetylene	Chlorine, copper, bromine, fluorine, mercury, silver
Acrolein	Non-oxidizing mineral acids
Acrylic acid	Aromatic amines
Acrylonitrile	Caustics
Alcohols	Aliphatic amines, caustics, isocyanates, nitric acid, sulfuric acid
Aldehydes	Amines, ammonia, caustics, nitric acid, sulfuric acid
Alkali and alkaline earth metals	Carbon tetrachloride or other chlorinated hydrocarbons, carbon dioxide, halogens, water
Alkyl nitrates	Non-oxidizing mineral acids
Allyl alcohol	Isocyanates
Ammonia (anhydrous)	Bromine, calcium hypochlorite, chlorine, hydrofluoric acid (anhydrous), iodine, mercury
Ammonium nitrate	Acids, chlorates, flammable liquids, nitrites, powdered metals, sulfur, finely divided organic or combustible materials
Aniline	Hydrogen peroxide, nitric acid
Arsenical materials	Any reducing agent
Azides	Acids
Bromine	Acetylene, ammonia, benzene, butadiene, butane, hydrogen, finely divided metals, methane, propane, sodium carbide, turpentine
Calcium oxide	Water
Carbon(activated)	Calcium hypochlorite, all oxidizing agents
Carbon tetrachloride	Sodium
Chlorates	Ammonium slats, acids, powdered metals, sulfur finely divided organic or combustible materials
Chromic acid and chromium trioxide	Acetic acid, naphthalene, camphor, glycerol, alcohol, flammable liquids
Chlorine	Ammonia, acetylene, butadiene, butane, methane, propane, hydrogen, sodium carbide, benzene, finely divided metals, turpentine
Chlorine dioxide	Ammonia, methane, phosphine, hydrogen sulfide
Copper	Acetylene, hydrogen peroxide
Crotonaldehyde	Non-oxidizing mineral acids
Cumene hydroperoxide	Acid (organic or inorganic)
Cyanides	Acids
Cyclohexanone, cyclohexanol mixture	Isocyanates
Decaborane	Carbon tetrachloride and some other halogenated hydrocarbons
Dichloromethane	Alkali metals (lithium, sodium, potassium), strong bases, strong oxidizers (perchlorates, peroxides, permanganates, chlorates, nitrates, chlorine, bromine, fluorine), magnesium, aluminum
2,4-Dichlorophenoxyacetic acid, triisopropanolamine salt solution	Nitric acid
Dimethyl hydrogen phosphite	Non-oxidizing mineral acids, organic acids
Dimethyl naphthalene sulfonic acid, sodium salt solution	Isocyanates
Ethers	Caustics, ammonia, amines
Ethylenediamine	Ethylene dichloride
Ethylidene norbonene	Aliphatic amines, , alkanolamines, ammonia, caustics, mineral acids
2-Ethyl-3-propylacrolein	Non-oxidizing mineral acids
Fish oil	Sulfuric acid
Flammable liquids	Ammonium nitrate, chromic acid, hydrogen peroxide, nitric acid, sodium peroxide, halogens
Fluorine	Everything
Formaldehyde ca. 37 % in water (methanol stabilized)	Strong oxidizers, isocyanates, acids, amines, strong bases, phenol, peroxides, acid anhydrides
Formaldehyde (over 50 %) in methanol (over 30 %)	Isocyanates
Formalin solution, neutral buffered, 10 %	Strong bases, acids, oxidizing agents, alkali metals amines, acid chlorides, acid anhydrides, reducing agents, peroxides, isocyanates, phenol
Formic acid	Furfural alcohol
Furfuryl alcohol	Non-oxidizing mineral acids, formic acid
Hydrocarbons (such as butane, propane, benzene, pentene)	Fluorine, chlorine, bromine, chromic acid, sodium peroxide, hydrocyanic acid, nitric acid, alkali, oxidizing agents
Hydrofluoric acid (anhydrous)	Ammonia (aqueous or anhydrous)
Hydrogen peroxide	Copper, chromium, iron, most metals and their salts, alcohols, acetone, organics, aniline, nitromethane, combustible materials
Hydrogen sulfide	Fuming nitric acid, oxidizing gases
Hydrazine	Nitric acid, hydrogen peroxide, and other oxidants
Hypochlorites	Acids, activated carbon
Iodine	Acetylene, ammonia(aqueous or anhydrous), hydrogen
Isocyanates	Acids, caustics, ammonia, amines, amides, alcohols, aqueous solutions, caprolactam solution, glycol ether

Isophorone	Alkanolamines
Ketones	Sulfuric acid, nitric acid, aliphatic amines
Magnesium chloride solution	Sulfuric acid, nitric acid, caustics, ammonia, isocyanates
Mercury	Acetylene, ammonia, fulminic acid
Methacrylonitrile	Caustics
Methyl tert-butyl ether	Non-oxidizing mineral acids
Nitrates	Sulfuric acid
Nitric acid*	Acetic acid, aniline, chromic acid, hydrocyanic acid, hydrogen sulfide, flammable liquids, flammable gases, copper, brass, any heavy metals, ORGANICS
Nitrites	Acids
Nitriles	Sulfuric acid
Nitroparaffins	Inorganic bases, amines
o-Nitrophenol	Ammonia, amines, amides, caustics, nitric acid, sulfuric acid
Oxalic acid	Mercury, silver
Oxygen	Flammable liquid, grease, hydrogen, oils
Perchloric acid	Acetic anhydride, alcohol, bismuth and its alloys, grease, oil, paper, wood
Peroxides, organic	Acids (organic or mineral), avoid friction
Potassium	Carbon dioxide, carbon tetrachloride, water
Potassium chlorate	Sulfuric and other acids
Potassium perchlorate(and other perchlorates)	Sulfuric and other acids
Potassium permanganate	Benzaldehyde, ethylene glycol, glycerol, sulfuric acid
Selenides	Reducing agents
Silver	Acetylene, ammonium compounds, oxalic acid, tartaric acid, fulminic acid
Sodium	Carbon tetrachloride, carbon dioxide dichloromethane, water
Sodium azide	Acids, acid chlorides, halogenated hydrocarbon, metals
Sodium hydrogen sulfide, sodium carbonate solution	Ammonia, aliphatic amines
Sodium hydrogen sulfide, ammonium sulfide solution	Ammonia, aliphatic amines
Sodium hydrosulfide	Ammonia, aliphatic amines
Sodium nitrite	Ammonium nitrate and other ammonium salts
Sodium peroxide	Acetic anhydride, carbon disulfide, ethyl or methyl alcohol, benzaldehyde, ethylene glycol, ethyl acetate, furfural, glycerine , glacial acetic acid, methyl acetate
Sodium polyacrylate solution	Nitric acid
Sodium silicate solution	Nitric acid
Sodium thiocyanate	Acids
Sulfides	Acids
Sulfuric acid	Potassium chlorate, potassium perchlorate, potassium permanganate
Tellurides	Reducing agents
1,1,1-Trichloroethane	Oleum
Trichloroethylene	Caustics
Triethyl phosphite	Non-oxidizing mineral acids, organic acids
Trimethyl phosphite	Non-oxidizing mineral acids, organic acids
1,3,5-trioxane	Non-oxidizing mineral acids, organic acids

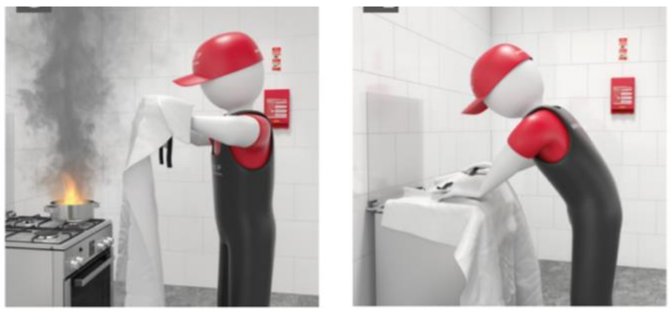
Flame Retardant Blanket

Flame Retardant Blanket: A non-reusable piece of flexible material, intended for use by a single person, which is intended to extinguish small fires by smothering the flames.

Operating Instructions:

- If it is safe, eliminate the heat source;
- Remove the blanket from the box, pulling on the ribbons;
- Pick up the blanket in order to protect your hands, face and torso;

- Approach by lifting the blanket to protect yourself;
- Cover the entire perimeter of the fire;
- Remove the blanket only when the fuel has cooled down.



Symptoms of breakdown

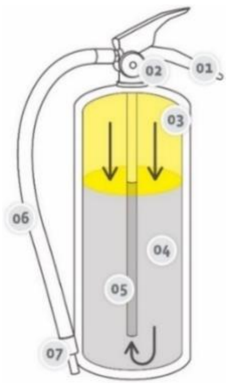
- Handles not visible or not aligned.
- Attachment to the bracket damaged.
- Dirty or broken blanket .

Safety Procedures

- Access to the blanket must be kept clear and unobstructed.
- Ensure that the blanket is properly secured to its support.
- Ensure that the upper part of the blanket is about 1.70 meters from the floor.
- If the blanket is to be used, ensure that it is subsequently replaced.

ABC Powder Fire Extinguisher

ABC Powder Fire Extinguisher: suitable for extinguishing fires resulting from the combustion of class A, B and C materials (e.g. wood, cardboard, fabrics, petrol, diesel, butane, propane, etc)



Legenda
1. Manípulo
2. Manómetro
3. Gás propulsor
4. Agente Extintor
5. Tubo de Saída
6. Mangueira
7. Boquilha

Operating Instructions:

- ✓ Check that the extinguisher is suitable for the type of fire;
- ✓ Carry the extinguisher to the fire source;
- ✓ Remove the safety pin;
- ✓ Perform a small test flush;
- ✓ Point the nozzle at the base of the flames at a distance of about 3 m;
- ✓ After extinguishing the fire, place the extinguisher lying on the ground.

Symptoms of breakdown

- ❖ Absence of dowel.
- ❖ Fire extinguisher damaged.
- ❖ Rupture in the hose.
- ❖ Pressure gauge in the red position.
- ❖ Damaged handle.

Safety Procedures

- ❖ Access to the extinguisher must be kept clear and unobstructed.
- ❖ Always keep extinguisher maintenance up to date.
- ❖ Do not change the location of fire extinguishers.
- ❖ Keep a safe distance while extinguishing the fire.
- ❖ Do not try to fight the fire if you are not safe.

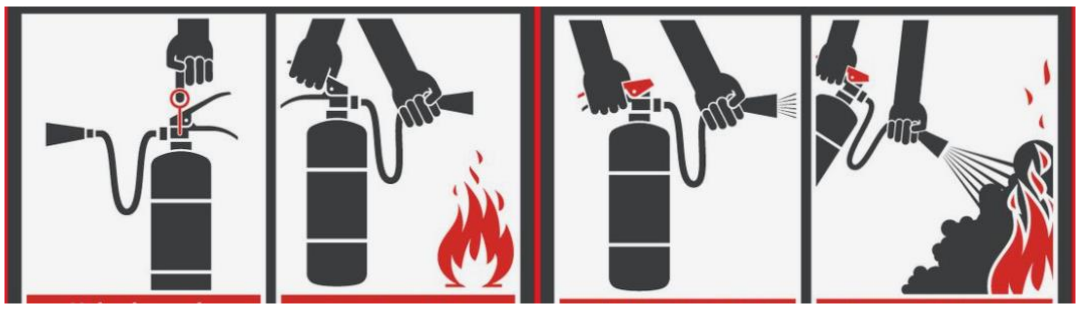
[next page: CO$_2$ fire extinguisher]

CO_2 fire extinguisher

CO_2 fire extinguisher: Suitable for use in electrical equipment fires.

Operating Instructions:

- ✓ Check that the extinguisher is suitable for the type of fire;
- ✓ Carry the extinguisher to the fire source;
- ✓ Remove the safety pin;
- ✓ Pick up the diffuser through the handle;
- ✓ Perform a small test flush;
- ✓ Point the diffuser at the base of the flames;
- ✓ After extinguishing the fire, place the extinguisher lying on the ground.
- ✓



Symptoms of breakdown

- ❖ Absence of dowel.
- ❖ Fire extinguisher damaged.
- ❖ Broken diffuser or hose.
- ❖ Damaged handle.

Safety Procedures

- ❖ Access to the extinguisher must be kept clear and unobstructed.
- ❖ Always keep extinguisher maintenance up to date.
- ❖ Do not change the location of fire extinguishers.
- ❖ Keep a safe distance while extinguishing the fire.
- ❖ Do not try to fight the fire if you are not safe.

Spill Containment and Removal Kit

Liquid spill containment kits consist of:

- ✓ 1 x Bucket or bag with sorbent material (bentonites, sepiolite or other minerals);
- ✓ 1 x Filter mask containing ABEK type filters (Organic gases and vapours - boiling point > 65 °C; Inorganic Gases and Vapors - excluding Carbon Monoxide; Sulphur dioxide and other acid gases and vapors; Ammonia and organic ammonia derivatives), preferably of class P3 (high capacity). This mask is suitable for substances that are clearly perceptible through odor or taste!;
- ✓ 1 x chemical goggles (EN 166 standard);
- ✓ 1 x pair of chemical protection gloves (EN 374 standard);
- ✓ 1 x broom and catcher;
- ✓ 1 x empty bucket, with lid, for collecting and disposing of spillage.

Scope of use

For spills of hazardous liquid substances or mixtures (see exceptions in the notes below), the size of which does not allow them to be quickly remedied with absorbent paper and its disposal as hazardous solid waste.

Spills of **more than 5 liters** are major accidents, and the area must be isolated and the Security Post should be immediately informed.

Common Spills

- Concentrated acids and bases (main hazards: release of toxic fumes, corrosion and slippery floor);
- Flammable solvents (main hazards: risk of fire and release of toxic vapours);
- Highly toxic liquids and/or nauseating smells (e.g. pyridine and aniline).

IMPORTANT NOTE 1: Hydrofluoric acid spills require special neutralizing sorbents, as this acid reacts with common sorbents.

IMPORTANT NOTE 2: The sorbent material available in the Kits is not effective for mercury spills.

Recommended mode of action

i) If the liquid releases toxic or flammable vapors, remove all occupants from the room(s) and close the doors;
ii) If the spill happens outside a laboratory, for example in a corridor or in the freight elevator, warn all people in the vicinity to move away;
iii) If in doubt, treat the spill as being toxic and flammable;
iv) Open windows only if they face outwards. Put on the mask (in case of toxic product), goggles and safety gloves, preferably outside the site of the spill;
v) You should only act if there is someone is around and able to check on you by maintaining eye contact, following the course of the action;
vi) Enter the site and turn off possible sources of ignition in case of flammables (switch off laboratory boards if possible);
vii) Circumscribe the spill by pouring sorbent around it;
viii) If vapors are released, pour sorbent also over the spilled liquid to stop the vapor (see Fig.1);
ix) After the liquid is completely absorbed, collect the mixture into the disposal bucket using the gloves, the broom and the catcher;
x) If appropriate, neutralise the liquid inside the fume hood before closing the bucket and disposing of it as hazardous waste.

Figure 1. Sequence of images showing the delimitation, covering and collection of the spilled liquid.

Filtering Mask

The filtering mask is the most important protective element in case of release of toxic vapors, and it is very important to know its main characteristics and have prior training in its use.

Failure to follow the instructions may adversely affect the user's health (at the limit, lethal diseases or permanent disability) | It is extremely important to have a prior notion of the hazards of the liquids handled!

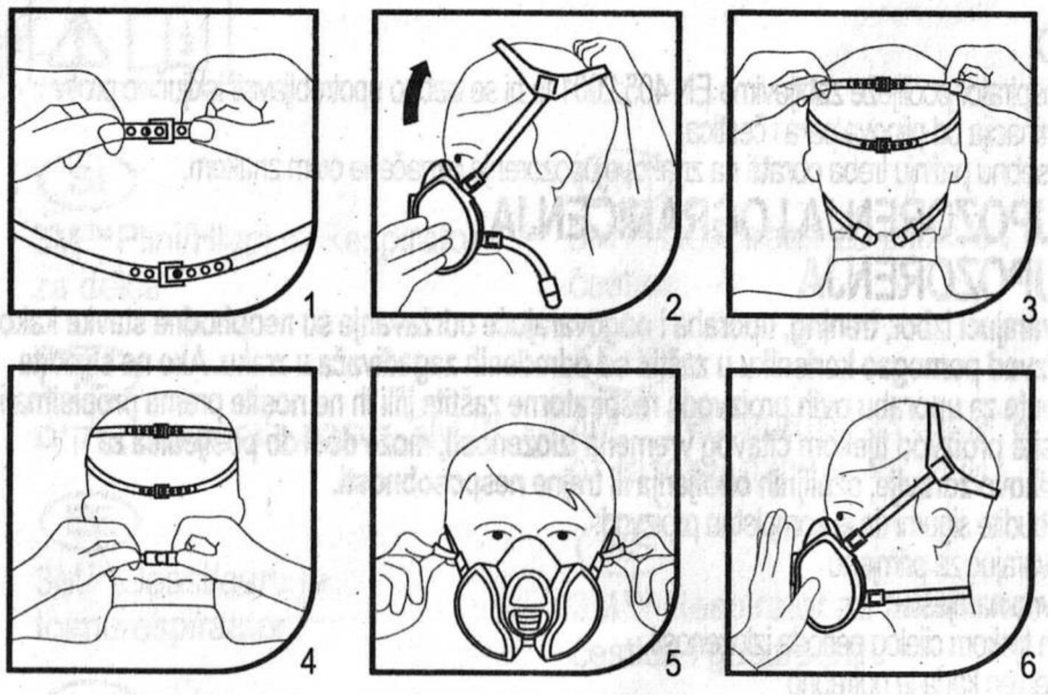
The equipment that integrates the spill containment kits is called a respirator, 3M model 4279+ (Figure 2), and it must be ensured that it is suitable for the job, fits correctly, is used throughout the exposure period and is replaced whenever necessary.

Figure 2. Respirator included in the spill containment and removal kit.

Limitations of Use:

1. Leave the area immediately if:
    - i) Breathing becomes difficult or there is an increase in breathing resistance;
    - ii) Feeling dizzy;
    - iii) Smelling or tasting contaminants or irritation occurs.
2. Never alter or repair this product.

Adjustment Visual Instructions:



Checking the seal:

1. Do a positive pressure adjustment test: place the palm of your hand on the cover of the exhalation valve and exhale carefully; If the facepiece swells slightly and there is no air leakage between the face and the facepiece, a correct fit has been achieved.

2. If an air leak is detected, reposition the mask on the face and/or readjust the tension of the head harness elastics to eliminate the leakage. (The use of a beard or other facial features can prevent a good seal.)

3. If a correct fit is not achieved, do not enter the contaminated area.

Cleaning and disinfection:

This equipment is reusable. View the cleaning, disinfecting and storage instructions provided by the manufacturer.

OCCURRENCE REPORT FORM (ACCIDENT OR NEAR MISS)

Identification of the person(s) involved

Names, categories (Prof./Researcher/Stud./etc.), contacts (phone/email):

Location of occurrence (Department/laboratory or other):

Laboratory Safety Officer or Supervisor:

Witnesses

Names, categories (Prof./Researcher/Stud./etc.), contacts (phone/email):

Description of the occurrence (describe the sequence of events, including date and time; attach photos, schematics, and additional sheets, if necessary)

Causality factors (describe conditions or practices that may have contributed to this occurrence; attach additional pages if necessary)

Corrective actions (in your opinion, what reasonable actions could eliminate or reduce the risk of occurrences like this? Attach additional pages if necessary)

RSL or Supervisor Signature: Date:

Comments from the Departmental Safety Committee:

Signature: Date: